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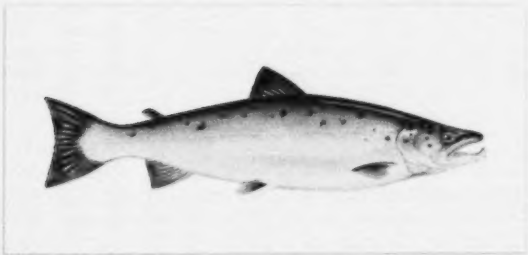
Science Advisory Report 2018/041

Maritimes Region

REVIEW OF THE SCIENCE ASSOCIATED WITH THE INNER BAY OF FUNDY ATLANTIC SALMON LIVE GENE BANK AND SUPPLEMENTATION PROGRAMS



Line drawing of a male adult Atlantic Salmon (adapted from Amiro 2003).

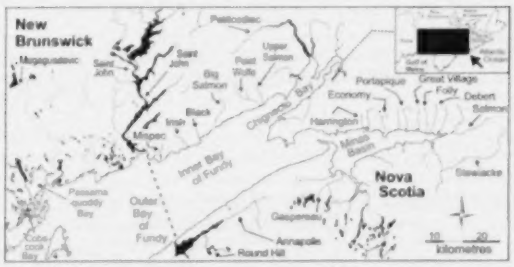


Figure 1. Location of inner Bay of Fundy rivers, including those referenced in the review (blue font).

Context:

The Designatable Unit of Inner Bay of Fundy (iBoF) Atlantic Salmon, once consisting of tens of thousands of adult returns, began to decline in the late 1980s. Population size reductions continued all through the 1990s, and in the year 1999, less than an estimated 250 adults are believed to have returned to the approximately 50 rivers of the inner bay. To prevent the imminent extirpation of this phenotypically and genetically distinct group of Atlantic Salmon, some of the last remaining juveniles were captured between 1998 and 2001, and transferred to biodiversity facilities in the Maritimes Region of Fisheries and Oceans Canada for captive breeding and rearing. This Live Gene Banking (LGB) program, and associated juvenile and adult supplementation efforts, has been in operation for a little over 15 years (across 3 Salmon generations) and there is little evidence of progress towards the re-establishment of self-sustaining populations.

Maintenance of small populations in captive or semi-captive environments is not without risks, some of which could potentially impact the efficacy of present or future population recovery efforts. Possible concerns include (i) loss of genetic variation, (ii) accumulation of inbreeding and associated inbreeding depression, (iii) random changes in overall allele frequency distributions and loss of local adaptation, and (iv) adaptation to captivity and associated loss of fitness in the wild.

The purpose of the review meeting was to assess (a) the science associated with the development and ongoing modification of the LGB program, (b) reported rates of drift-induced genetic change (see i to iii above) in iBoF Salmon managed under the program, c) effects of various program variables on fitness-related traits both in captivity and in the wild, d) rates and direction of change in a suite of monitored phenotypic traits over time, e) possible genetic changes in, primarily, the Big Salmon River population resulting from recent increases in introgressive hybridization involving both non-native wild and farm Salmon, and f) the numbers and origins of adult returns to iBoF rivers in recent years.

This Science Advisory Report is from the June 13-16, 2017, Review of the Inner Bay of Fundy Atlantic Salmon Science Associated with the Live Gene Bank. Additional publications from this meeting will be posted on the Fisheries and Oceans Canada (DFO) Science Advisory Schedule as they become available.

Canada

SUMMARY

- The inner Bay of Fundy (iBoF) Atlantic Salmon Live Gene Bank (LGB) program supports Objective 1 in the Inner Bay of Fundy Atlantic Salmon Recovery Strategy (conserve iBoF Salmon genetic characteristics and re-establish self-sustaining populations to iBoF rivers), and is a part of the larger iBoF Salmon recovery program that also includes hatchery supplementation involving the release of juveniles and adults into native river habitat.
- This review provides an assessment of the LGB program following 3 generations (i.e., approximately 15 years) of iBoF Salmon population maintenance activities, which will inform the development of an updated 5-year plan for the LGB program.
- Results are summarized below for each of the five Terms of Reference for this review, followed by a summary of considerations for the future management of the LGB program. Additional program results and recommendations are contained in the text of this report.

Evaluate Success of Conserving Genetic Characteristics of the iBoF Salmon Population Across Three Generations of Captive Breeding and Rearing

- Sufficient information concerning both genetic variability and fitness is available to evaluate this objective for the Stewiacke River population only.
- The original collection of 1,029 Stewiake Salmon (i.e., the group of parr collected from the wild between 1998 and 2001, which include the primary founders) exhibited very high levels of family structuring.
- Levels of genetic variation in the present day Stewiake population may have been markedly higher, and levels of inbreeding lower, if initial founder collections were initiated even two years earlier.
- High variability in the genetic contributions of the parents of the originating founders has resulted in elevated rates of loss of founder parent variation (e.g., founder parent alleles and founder parent genome equivalents) over time, and higher than anticipated rates of accumulation of inbreeding in subsequent generations.
- Overall, variance in family size was lower for captive-reared versus wild-exposed groups from a given brood year.
- Overall, rates of loss of molecular genetic variation between the Stewiacke founder and second generation Salmon were low, though some slight reductions were observed.

Investigate Levels and Origins of Inbreeding in iBoF Salmon

- The estimated effective number of Stewiacke breeders under the wild-exposed offspring management regime is probably sufficiently large that ongoing rates of loss of genetic variation due to random genetic drift, and rates of accumulation of inbreeding, are not expected to be high.

Evaluate the Effects of the Overall Program on Fitness-related Traits in iBoF Salmon

- There was no evidence for possible effects of parental early juvenile rearing environment (captive versus wild) on early offspring survival in the wild.
- In some years, the parental early juvenile rearing environment (captive versus wild) may have influenced size-at-age of offspring in their first year in the wild, with offspring of wild-exposed Salmon exhibiting greater length and weight compared to offspring of exclusively captive-reared parents.

- No evidence was found for possible cumulative negative effects of ancestral (parent and grandparent) early juvenile captive rearing environments on early offspring survival in the wild.
- In some years, a possible negative influence of increasing number of generations of captive breeding and rearing on survival in the wild was observed and, although the effect was small, the number of generations across which comparisons were possible was very limited.
- No association between measures of molecular genetic variation or pedigree inbreeding and either survival in the wild or the incidence or percentage of fry deformities was observed.
- Female parent age, but not male parent age, was found to have a large effect on multiple offspring performance traits, including survival in the wild from release to Age 1, and this effect was consistent across multiple years; part of this effect appears to be due to egg size, but female parent age was also associated with increased survival independent of egg size in some years.
- Generally, most monitored traits in the Stewiacke population did not show any discernible trends over the course of the program.

Assess the Status of Atlantic Salmon in the iBoF Designated Unit based on DFO Information and, Where Possible, Evaluate the Efficiency and/or Appropriateness of the Different Release Strategies

- The observation that juvenile Salmon are no longer present, or if present are at extremely low densities, indicates the lack of returning truly wild iBoF Salmon and that iBoF Salmon would likely be extirpated without the LGB program.
- Based on the genetic analysis, about 24% of the small Salmon returns on the Big Salmon River and the majority of the adult returns to White Rock Dam on the Gaspereau River have been the direct result of the LGB program.
- On the Big Salmon River, the mean annual smolt production from 2003 until 2016 has been approximately 13,400 smolts. Over this time period, the annual contributions by origin breakdown is as follows: adult spawners have contributed 4,729 fish, LGB unfed fry releases have contributed 4,646 fish and adipose-clipped LGB fall and spring parr releases have produced 4,034 fish.
- Both Gaspereau and Big Salmon rivers remain well below conservation egg requirement due to poor marine survival. The smolt to small Salmon return rate from the combined group of adult spawners, LGB fry, and LGB parr has been consistently below 1% and has averaged 0.32% from 2002 to 2016 on the Big Salmon River.
- The mean smolt to small Salmon return rate for the smolts that originated from LGB fry is 0.20%, which is about three times better than the mean return rate for LGB origin smolts released as parr.

Provide Information Concerning Introgression of Non-native Wild and Aquaculture Genetic Material into iBoF Populations

- Genetic structuring between outer Bay of Fundy and Big Salmon River Salmon appears to be decreasing over time. Results from several population genetic analyses are all consistent with extensive and ongoing gene flow from non-local (likely outer Bay of Fundy) sources into the Big Salmon River gene pool, and this could represent a risk to the conservation of iBoF genetic characteristics in the Big Salmon River LGB population.

- European farm Salmon ancestry, the proximate source of which appears to be the local (Bay of Fundy/Gulf of Maine) industry, has been detected in Salmon collected from the Upper Salmon and other iBoF rivers. European farm escapes, or their European farm/North American farm hybrid offspring, appear to have been spawning in the iBoF during the period spanning 1997 to 2012, including during all or most years within this period.
- Analyses indicate that farm Salmon escapes exhibiting European ancestry have successfully reproduced in several iBoF rivers and appear to have spawned with endangered iBoF Atlantic Salmon.
- European farm/North American hybrids may exhibit reduced early juvenile survival relative to pure Stewiacke parents, in wild Stewiacke River habitat.

Considerations for Future Management of the LGB Program

- The likely number of adult returns to individual rivers in the iBoF (0 to 10 in most years with the exception of the Big Salmon River) is probably too low for natural selection to be effective at maintaining or increasing survival under current and possibly changing marine conditions. For natural selection to impact or improve smolt-to-adult survival, a sufficient number of smolt must exit a given iBoF river for a small to moderate number of adults to return to either reproduce in native river habitat, or be incorporated into LGB populations directly as spawners. This may require increasing the number of fish released from the LGB program.
- There are several possible paths to maintaining large effective population size in iBoF LGB programs, each involving variable levels of expenditure of different resources; an optimal approach can be selected based on scarcity of each resource at a particular point in time.
- If a shift in focus of the iBoF Recovery program from maintenance of genetic variation to re-establishment of wild self-sustaining populations were to be considered, the extent to which resources ought to be redirected should reflect both costs (e.g., reduced efficacy of conserving genetic variation) and benefits (e.g., the likelihood of making progress toward re-establishing self-sustaining populations, given current marine conditions).

INTRODUCTION

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) identified the inner Bay of Fundy (iBoF) Atlantic Salmon assemblage as a Designatable Unit (DU) and assessed this population as Endangered in May 2001 (COSEWIC 2006). This population was included as Endangered on Schedule 1 of the *Species at Risk Act* (SARA) when the *Act* was passed in 2002. In 1998, prior to listing under SARA, population trends observed in several rivers of the iBoF prompted the collections of juveniles to be reared in the biodiversity facilities in the Maritimes Region (Mactaquac, Mersey, and Coldbrook Biodiversity Facilities) effectively initiating the present-day Live Gene Bank (LGB) programs. The objective of the LGB program is to use captive breeding and rearing technologies to conserve genetic characteristics of iBoF Salmon and maintain populations until recovery can occur (DFO 2008a). In 2008, the Recovery Potential Assessment (RPA) forecasted that this population would be extinct without the support of the LGB program (DFO 2008b; Gibson et al. 2008).

Several evaluations have been conducted in recent years to assess the scientific merit of the LGB program (O'Reilly and Harvie 2010; O'Reilly and Doyle 2007; O'Reilly and Kozfkay 2014). However, a comprehensive analysis and review of the contribution of the LGB program (and associated supplementation activities) to recovery objectives of the iBoF population over the lifetime of the program had not been undertaken. Therefore, DFO Maritimes Science requested

the evaluation of iBoF Salmon science with respect to all LGB and related activities. The intent of this review was to provide an assessment of the LGB program following 3 generations (i.e., approximately 15 years) of iBoF Salmon population maintenance activities as population recovery has yet to occur. This review will inform the development of an updated 5-year plan for the LGB program.

The purpose of the meeting was to evaluate the contribution of the LGB program and associated supplementation in achieving the key objectives of the iBoF Salmon Recovery Strategy to conserve iBoF Salmon genetic characteristics and re-establish self-sustaining populations to iBoF rivers. More specifically, the objectives of the meeting were to:

- evaluate the success of conserving genetic characteristics of the iBoF Salmon population across three generations of captive breeding and rearing,
- investigate levels and origins of inbreeding in iBoF Salmon,
- evaluate the effects of the overall program (multiple generations of captive breeding and rearing), and specific management strategies employed within, on fitness-related traits in iBoF Salmon, and
- assess the status of Atlantic Salmon in the iBoF DU based on DFO information, and where possible, to evaluate the efficiency and/or appropriateness of the different release strategies.

In addition, recent information concerning introgression of non-native wild and aquaculture genetic material into iBoF populations was tabled for consideration in the evaluation of LGB activities.

The iBoF LGB program is part of the larger iBoF Salmon recovery program that also includes hatchery supplementation involving the release of juveniles and adults into native river habitat. Live Gene Bank operations are depicted above the broken red line in the schematic below (Figure 2).

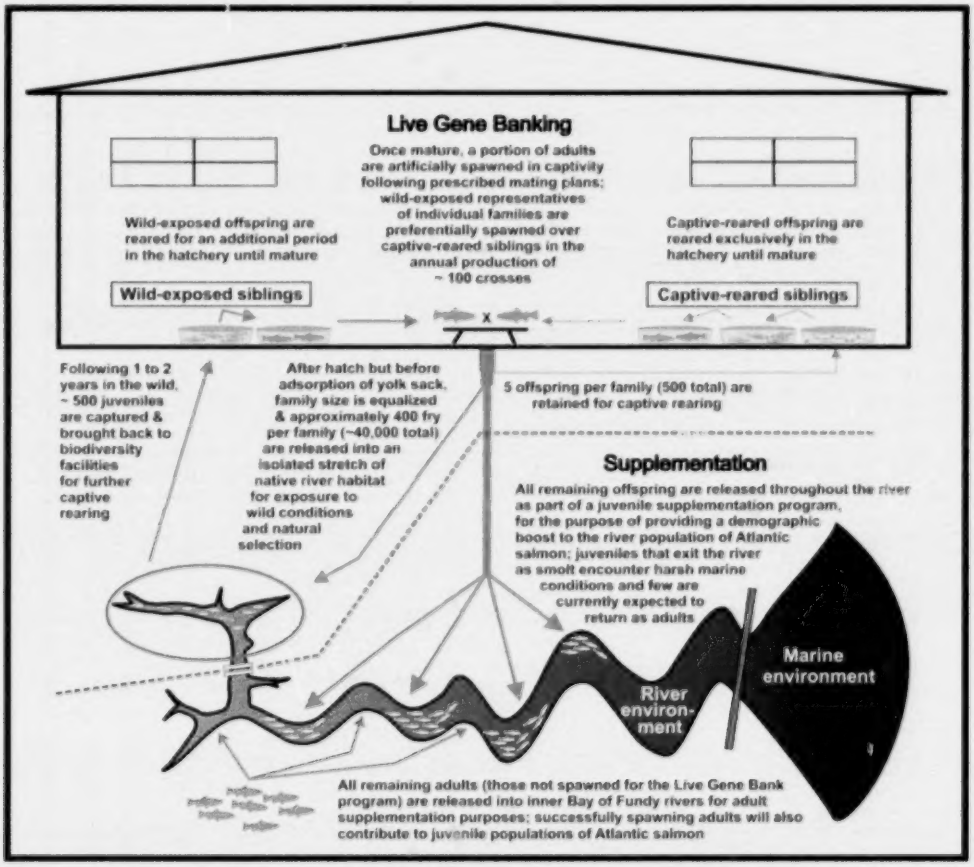

Live Gene Banking
Once mature, a portion of adults are artificially spawned in captivity following prescribed mating plans; wild-exposed representatives of individual families are preferentially spawned over captive-reared siblings in the annual production of ~ 100 crosses
Wild-exposed offspring are reared for an additional period in the hatchery until mature
Captive-reared offspring are reared exclusively in the hatchery until mature
Wild-exposed siblings
Captive-reared siblings
Following 1 to 2 years in the wild, ~ 500 juveniles are captured & brought back to biodiversity facilities for further captive rearing
After hatch but before adsorption of yolk sack, family size is equalized & approximately 400 fry per family (~40,000 total) are released into an isolated stretch of native river habitat for exposure to wild conditions and natural selection
5 offspring per family (500 total) are retained for captive rearing
Supplementation
All remaining offspring are released throughout the river as part of a juvenile supplementation program, for the purpose of providing a demographic boost to the river population of Atlantic salmon; juveniles that exit the river as smolt encounter harsh marine conditions and few are currently expected to return as adults
River environ-ment
Marine environment
All remaining adults (those not spawned for the Live Gene Bank program) are released into inner Bay of Fundy rivers for adult supplementation purposes; successfully spawning adults will also contribute to juvenile populations of Atlantic salmon

Figure 2. Schematic of the current Stewiacke River inner Bay of Fundy conservation program, including Live Gene Banking (above red dotted line) and supplementation (below red dotted line) components.

Prior to maturation, Stewiacke (STW) River Atlantic Salmon from a given brood year or year class are genotyped and pedigreed. Spawners are selected from the larger group of available mature Salmon, and males and females are paired following prescribed mating plans based on Ranked Mean Kinship (RMK) procedures. Once all the approximately 100 prescribed crosses are carried out, a small number of offspring (5 per family) are reared exclusively in captivity through to maturity (referred to as 'captive-reared' Salmon). A larger number of offspring (approximately 400 per family) are enumerated and released into an isolated tributary of the STW River (the Pembroke River) where they are exposed to natural selection for one to two years before being captured and brought back to the biodiversity facility for additional captive rearing (referred to as 'wild-exposed' Salmon). Before maturation, both captive-reared and wild-exposed Salmon are genotyped, pedigreed, and spawned (as described above) in the production of the next generation of STW Salmon; when both captive-reared and wild-exposed individuals are available from a given family, spawning preference is given to the wild-exposed sibling. These operations collectively represent the LGB component of the STW River iBoF conservation program. The main objective of iBoF LGB program is to conserve within-population genetic variation across multiple cycles of captive breeding and rearing, while attempting to minimize, to the extent possible, adaptation to captivity and cumulative loss of fitness in the wild. Juveniles from the approximately 100 crosses carried out each year that are not managed under either the 'captive-reared' or 'wild-exposed' regimes described above are

released throughout the river for juvenile supplementation purposes (see Figure 2, below red dotted line). Similarly, adults not spawned in the LGB program are released throughout the STW River for adult supplementation purposes.

Stewiacke River Atlantic Salmon are currently spawned for the LGB program in their 5th year, but a large proportion (>70%) of both captive-reared and wild-exposed individuals first mature in their 4th year. Mature, Age 4 Salmon are therefore also spawned, but only for supplementation purposes; their offspring are released throughout the STW River system, with the exception of the isolated Pembroke tributary discussed above. The objective of both juvenile and adult supplementation is to increase the size of the juvenile population in the STW River and, when marine conditions improve, the number of returning adults.

Similar LGB and supplementation initiatives are also underway for Salmon of the Gaspereau (GAK) and Big Salmon (BSR) rivers (Figure 1), though important differences among river-specific conservation programs exist. On the BSR River, a similar number of pedigree-informed crosses are carried out each year, and a comparable number of offspring are both reared exclusively in captivity (referred to as the 'captive-reared' Salmon) and released into an isolated tributary to be later captured as Age 1 and Age 2 parr (referred to in the BSR program as 'wild-exposed parr'). However, on the BSR, a Rotary Screw Trap (RST), located near the mouth of the river is operated every spring, and several hundred smolt are captured and brought back to captivity for rearing alongside exclusively captive-reared and wild-exposed parr (see Jones et al. 2018). A portion of these smolt are produced by LGB crosses, and they are referred to here as 'wild-exposed smolt', but many are produced 'in river' (in wild BSR habitat) by several different types of possible parents, including: a) released LGB-origin juveniles spawning as mature male parr; b) mature parr produced by previous 'in river' spawnings; c) LGB-origin adult returns (Salmon released as fry that subsequently out-migrated as smolt to later return as adults); d) wild native BSR adult returns; e) wild adult strays produced in other rivers; and f) farm adult Salmon escapes of either local Saint John River (SJR) or European origin. The availability and use (spawning) of Salmon captured as out-migrating smolt offers several potential advantages, including: i) increased duration of exposure of LGB-origin Salmon to wild river conditions and natural selection; ii) incorporation of genes produced by free-spawning LGB-origin Salmon into the BSR LGB gene pool; iii) incorporation of genes that survived current marine conditions into the BSR LGB gene pool; and iv) introduction of potential new BSR founder genes into the LGB gene pool. However, because of this large potential source of non-pedigreed program Salmon (smolt produced 'in river' by non-genotyped LGB origin mature male parr and/or adult returns), and other characteristics unique to the BSR program (see O'Reilly et al. 2018), spawners are selected and paired using a process called Mean Kinship Assist (MKA) and individuals spawned for the LGB program at multiple ages. The collection and spawning of 'in river' produced smolt also necessitated the development of other program characteristics that differ from those described for the STW population. For example, non-spawned BSR adults (either captive-origin, wild-exposed, or wild-produced) are only released for adult supplementation purposes into non-BSR rivers on the Chignecto Bay side of the iBoF. This practice is intended to minimize breaks or discontinuities in the BSR pedigree ultimately associated with the collection and spawning of smolt obtained at the mouth of the BSR (see O'Reilly et al. 2018 for more details).

The program for the GAK population differs from both the STW and BSR programs described above. Although a small number of offspring from each family are also retained for exclusive captive rearing through to maturity (captive-reared Salmon), a parallel wild-exposed parr program as described above for STW and BSR populations is not currently being implemented. In other words, similarly sized groups of fry from each and every family are not released into an isolated tributary of the GAK for later recapture as Age 1 to 2 parr (although efforts are underway to duplicate this activity on the GAK). Instead, in early winter (just after eggs are

'shocked'), the number of surviving eggs from each cross is estimated volumetrically, and a generally similar number of eggs (determined volumetrically) from each family are set aside and combined for future release as unfed fry into tributaries above the White Rock Dam. The remaining eggs are combined for rearing several weeks post-hatch, for subsequent release into tributaries below the White Rock Dam. The numbers of fry from each family identified for release above the White Rock Dam is a function of estimated rearing capacity above the dam, and efforts to minimize, to some extent, variance in family size in this subgroup of program offspring (see O'Reilly et al. 2018 for more details). A small percentage of Salmon released above White Rock Dam are captured as out-migrating smolt using smolt traps operated at the surface bypasses at the dam, and they are transferred to the Coldbrook Biodiversity Facility for further captive rearing through to maturity (see Jones et al. 2018). These individuals have experienced 2 to 3 years in the wild, and they are referred to here as wild-exposed smolt. Because of the large number of fry involved, releases above White Rock serve two main purposes, river supplementation and gene banking. Fry released below White Rock are not sampled/collected later for re-integration into the program, and they are solely released for supplementation purposes. Genotyped GAK Salmon not spawned for LGB purposes are released above White Rock Dam for adult supplementation, as discussed above for STW River Salmon. Smolt collections obtained at White Rock Dam therefore also include 'in river' produced Salmon, offspring of unplanned spawnings involving mixtures of genotyped and non-genotyped (mature male parr) parents. Because of uncertainties in the pedigree, and characteristics unique to GAK Salmon (e.g., lower rates of maturation in year 4, and lower rates of maturation in year 5 if spawned in year 4), spawners are selected and paired using a process called Mean Kinship Analysis (MKA), and individuals spawned for the LGB program at multiple ages (see O'Reilly et al. 2018 for more details). The collection and spawning of 'in river' produced smolt obtained at White Rock Dam also resulted in the development of other program characteristics that differ from those described for the STW population. For example, non-genotyped adults are only released for adult supplementation purposes into non-GAK rivers on the Minas Basin side of the iBoF. This practice is intended to minimize breaks in the GAK pedigree ultimately associated with the collection and spawning of smolt obtained at the dam (see O'Reilly et al. 2018 for more details).

As outlined above, supplementation is a by-product of the LGB program for the 3 key rivers. In 2016, 876,844 unfed fry and 973 adults were released (STW 253,371 unfed fry and 795 adults, GAK 219,075 fry and 178 adults, and BSR 404,398 fry rivers). Non-targeted Salmon not prioritized for the pedigree based component of the LGB program are released into the tributary of origin (i.e. STW, GAK, or BSR), as well as into vacant habitat of other non-LGB iBoF rivers. In 2016, 374,280 unfed fry and 409 mature adults were released from the Coldbrook Biodiversity Facility into the following non-LGB rivers: Debert, Folly, Salmon (Colchester) and St. Croix while in New Brunswick all non-targeted Salmon were released into the Petitcodiac system. In 2016 additional releases from the Mactaquac Biodiversity Facility included 50,000 unfed fry and 355 mature adults.

The RPA forecasted that this population would be extinct without the support of the LGB program. The LGB program is maintaining populations in a few rivers, but high marine mortality continues to prevent efforts to re-establish wild self-sustaining populations of iBoF Salmon.

ANALYSIS

Results of Analyses of Indicators of Program Efficacy

Results reported below involving assessments of rates of genetic change over time, and assessments of survival/growth effects of different management regimes employed in the past, pertain to the STW River population, the one index population for which sufficient information concerning both genetic variability and fitness is available to carry out the required analyses. Broodstock management programs for STW, BSR and GAK Salmon were similar (MKA) through to 2013.

Kinship Reconstruction, Ancestral Inbreeding, and Timing of Initiation of the iBoF LGB Program

Based on two independent analyses, assignment of founder generation (Generation Zero; G0) STW Salmon to half-sib (HS) groups is expected to be approximately 90% accurate. Pedigree assignment of G0 generation individuals in large true HS groups is expected to be markedly more accurate compared to assignment of individuals in small HS groups, and most G0 individuals were partitioned into large HS groups. Over half of all individuals incorrectly assigning to small HS groups likely belonged to other true small HS groups rather than to other medium or large HS groups. Given the nature of the majority of incorrect assignments, many errors are not expected to either contribute substantially to the level of inbreeding observed in the next generation, or result in many individuals that are genetically valuable not being incorporated into the LGB as founders. However, expected errors will likely have some degree of impact on Mean Kinship (MK) estimates and the efficacy of minimization of MK methods in reducing rates of loss of genetic variation over time.

The original collection of 1,029 STW River G0 Salmon (i.e., the group of parr collected from the wild between 1998 and 2001, which include the primary founders) exhibited very high levels of family structuring (Figure 3), with over 50% of all individuals observed in the 10 largest HS groups; the largest single HS group contained 117 individuals, over 10% of the entire collection. Family and genetic diversity declined markedly from the 1998 to 2001 collection years of STW River wild parr, with parr from later years contributing very few additional G0 HS groups that were not observed in collections from earlier years. Given (1) the trend towards increasing family diversity with sample collections obtained earlier rather than later, (2) expected reductions in family overlap between yearly sample collections obtained earlier rather than later (see O'Reilly et al. 2018), and (3) population demographics during the two decades prior to 1998 (see Figure 4), levels of family diversity would probably have been markedly higher had the 4 yearly STW River collections of wild parr been obtained even 2 years earlier (between 1996 and 1999 instead of between 1998 and 2001).

Given the timing of the observed major population bottleneck (1995-1999), high levels of ancestral cumulative inbreeding in the G0 generation of STW Salmon themselves are not expected; most inbreeding associated with this bottleneck would be expected to be delayed for several generations.

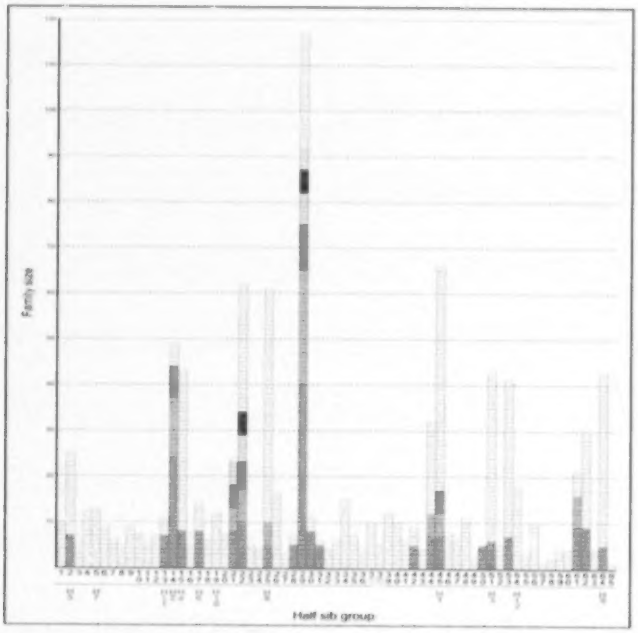


Figure 3. Distribution of the genotyped 1029 wild parr collected in 1998-2001 into 65 half-sib groups based on kinship reconstruction methods. Family size is given on the Y-axis, and half-sib group identification (ID) (in black font) on the X-axis. Each square represents a single individual. Large full-sib groups (consisting of 5 or more individuals) nested within half-sib groups are denoted by a continuous stretch of either blue or grey. Yellow squares denote individuals in small uncertain full-sib groups of 1 to 4 that may or may not be related. Below each arrow is the ID (in red font) given to the corresponding kin group in Herbinger et al. (2006).

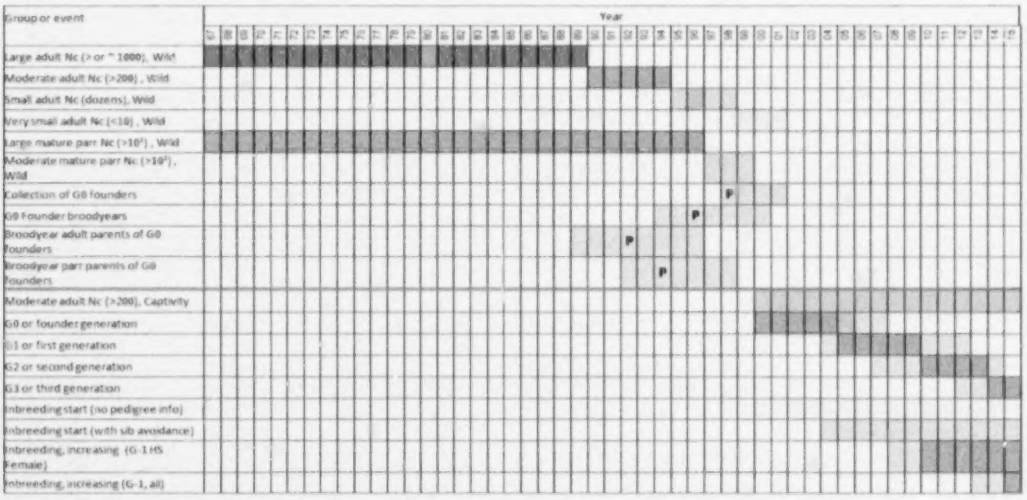

Group or event
Year
Large adult Nc (> or ~ 1000), Wild
Moderate adult Nc (>200), Wild
Small adult Nc (dozens), Wild
Very small adult Nc (<10), Wild
Large mature parr Nc (>10³), Wild
Moderate mature parr Nc (>10²), Wild
Collection of G0 founders
G0 Founder broodyears
Broodyear adult parents of G0 founders
Broodyear parr parents of G0 founders
Moderate adult Nc (>200), Captivity
G0 or founder generation
G1 or first generation
G2 or second generation
G3 or third generation
Inbreeding start (no pedigree info)
Inbreeding start (with sib avoidance)
Inbreeding, increasing (G-1 HS female)
Inbreeding, increasing (G-1, all)
P
P
P

Figure 4. Time frame over which the Stewiacke River adult and mature parr population sizes (adult Nc) remained at a given level, years of collection and 'in river' production of G0 founders, and expected timing of inbreeding resulting from the observed population bottleneck and family structuring. P denotes the primary year (1998) when a large portion of wild-origin founders (~40%) were collected, the brood year when most of the founders were produced (1996) and the year their parents, as either adults (1992) or mature male parr (1994), were likely produced. The double line separates wild and captive reproductive phases of the Stewiacke iBoF population. Adult Nc estimates were obtained from Gibson and Amiro (2003, Table 6).

Pedigree-based Estimates of G1 Founder Parent Contributions into the G2 Generation

The number of 2010-2014 STW LGB spawners descended from G1 founder parents (inferred parents of the G0 wild collected parr actually spawned in the LGB program) was very uneven (Figure 5). The most obvious source of much of this variability was the large difference in number of descendants produced by HS (red bars) versus full-sib (FS; blue bars) G1 founder parents types. Indeed, HS G1 founder parents on average left 56.6 G2 descendants, and FS G1 founder parents left 11.1 descendants. A total of 11 HS G1 founder parents produced 100 or more descendants, and another 18 produced between 50 and 100 descendants, far greater than the vast majority of FS G1 founder parents, which produced fewer than 25 descendants. Patterns of G1 founder parent genetic contributions paralleled that observed for founder parent descendants; levels were very high for a handful of G1 HS founder parents overall, especially compared to nearly all G1 FS founder parents (Figure 6). Although G1 founder parent retention also increased with the number of descendants and contributions, levels plateaued as they approached 1.0; additional founder parent contributions did not appreciably improve allele retention for these G1 HS individuals because values were already close to the maximum possible (Figure 7). On the other hand, allele retention was much less than 1.0 for most G1 FS parents and a portion of G1 HS parents. Over-representation of the genomes of a handful of G1 HS parents in G1 through G2 generation Salmon, limited retention of genes from G1 FS parents and some G1 HS parents. Were G0 Salmon collected even two years earlier, where founder groups may have been expected to have consisted of numerous small HS families with only a few FS families nested within (as observed in other larger nearby river populations of Atlantic Salmon analyzed), G1 founder contributions into future generations would likely have been much more even. This greater evenness in contributions would have been expected to have resulted in increased population-wide conservation of G1 founder parent genetic variation. In other words, high variability in the genetic contributions of STW G1 Salmon (parents of G0

founders) into future generations has resulted in elevated rates of loss of G1 founder parent variation (e.g., founder parent alleles and founder parent genome equivalents) over time. Based on population demographics between 1975 and 2000, and trends in family diversity observed in sample collections obtained between 1998 and 2001, levels of genetic variation in current STW LGB populations may have been markedly more diverse were initial founder collections initiated even two years earlier.

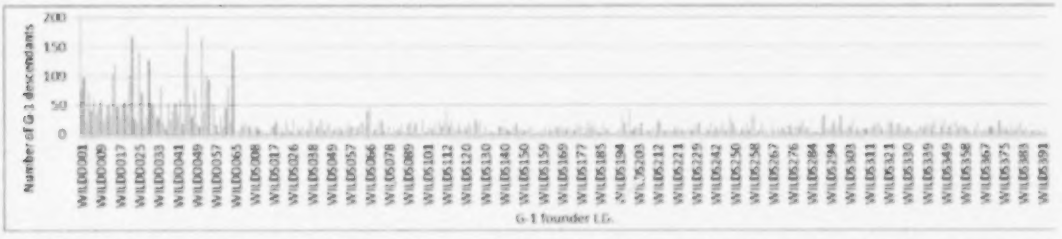


Figure 5. Number of G2 Stewiacke River descendants of individual G1 half-sib females (red bars) and G1 full-sib males (blue bars).

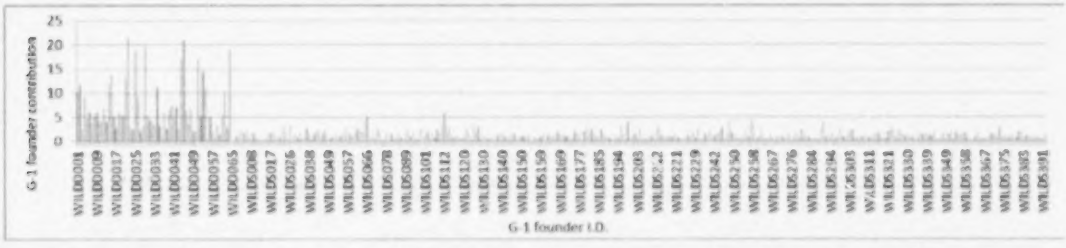


Figure 6. G1 half-sib (red bars) and full-sib (blue bars) founder parent contributions into the G2 generation of Stewiacke River spawners.

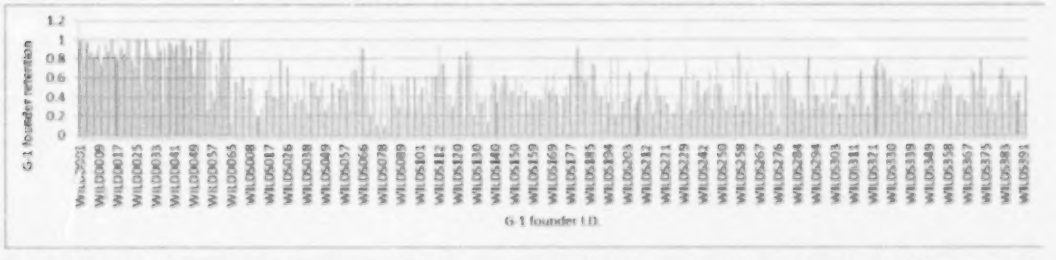


Figure 7. G1 half-sib (red bars) and full-sib (blue bars) founder parent allele retention in G2 generation of Stewiacke River spawners.

Observed Pedigree-inbreeding in Offspring Produced in the Years 2000-2015

Mean (across crosses) pedigree inbreeding of offspring of exclusively STW Salmon produced early in the program (2001-2003) was moderately high (F~0.003-0.005, Figure 8, blue bars). Inspection of inbreeding values for individual families indicates that this early inbreeding was due to a handful of crosses (3-5 each year) exhibiting very high levels of inbreeding (F=0.125), reflecting the presence of a single direct ancestor (an immediate parent) common between the dams and sires of these families. Although efforts were made to avoid crosses between FS and HS, occasional unintentional crosses between first-order relatives may have occurred either due to milt-carryover between spawnings, or changing kinship assignments associated with increasing program duration and cumulative increases in family sample size (O'Reilly et al. 2018). The very large size of some G0 HS groups increased the likelihood that male and female

parents of unplanned or incorrectly informed crosses would have shared a common G1 HS parent. Mean inbreeding was generally lower (F~0.001-0.002) between 2004 and 2009, when both family size was smaller and sibling avoidance was primarily based on parentage rather than kinship results. However, mean family or offspring inbreeding increased in later years, primarily starting in 2010 to F~0.004, and remained slightly elevated through to 2013. This increase in mean inbreeding levels coincides with a marked increase in the proportion of individual crosses (families) exhibiting levels of inbreeding greater than 0 (from approximately 10% or less in 2009 and earlier years, to approximately 30-50% in the years spanning 2010 to 2013). In over 90% of all instances, including 2015 which consisted of primarily G3 generation Salmon, ancestors common to dams and sires of the above crosses were G1 generation individuals, and almost all of these were putative HS females that produced large G0 HS groups. In other words, high variability in the genetic contributions of STW G1 Salmon (parents of G0 founders) into future generations has resulted in higher than anticipated rates of accumulation of inbreeding in the offspring of G2 and G3 generation Salmon. Based on population demographics between 1975 and 2000, and trends in family diversity observed in sample collections obtained between 1998 and 2001, levels of inbreeding may have been lower than observed today were initial founder collections initiated even two years earlier. However, this would not have prevented accumulation of inbreeding in future generations. Introgression of Economy (ECO), Great Village (GRV), and Debert (DEB) Salmon into the STW LGB has minimized accumulation of inbreeding slightly (Figure 8, black bars) but can lead to more inbreeding in the future, and needs to be monitored.

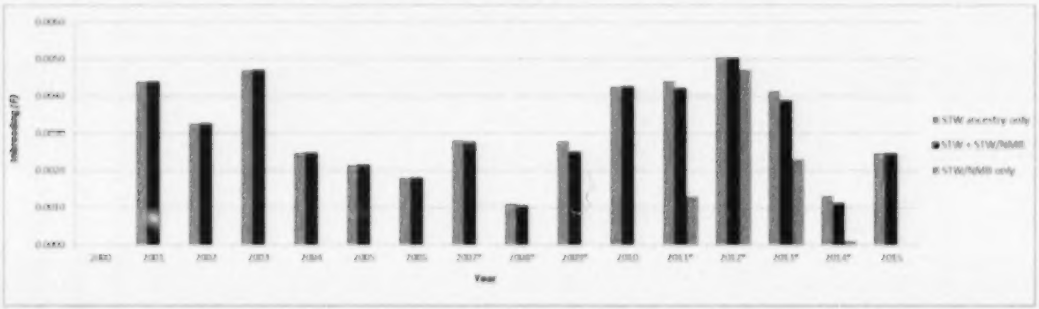


Figure 8. Average (across crosses) pedigree-based inbreeding of offspring of (1) Stewiacke (STW) ancestry only families (blue bars), (2) hybrid families (STW x North Minas Basin (NMB) or STW x STW/NMB or STW/NMB x STW/NMB, red bars), or (3) Stewiacke ancestry and hybrid families (black bars) for the years 2000 to 2015. Note, asterisks denote years where hybrid families were produced; levels of inbreeding for hybrid families produced in 2007, 2008, and 2009 were 0, so values for this class do not appear above the X-axis.

Observed Rates of Loss of Genetic Variation in Stewiacke River Salmon over Time

In the initial years, 2000-2003, founder parent Gene Diversity (GD) was approximately 0.9910-0.9915 but appeared to increase in 2004-2006 (a likely result of the mixing of non-overlapping sets of families in 1998 to 2001 collections) to the highest levels observed in the study, approximately 0.9940, before slowly declining across all future years to approximately 0.9920 by the end of the study. When tabulated by generation, however, this gradual reduction was not evident, as G0 to G2 Salmon all exhibited GD levels of approximately 0.9950. Number of parent Founder Genome Equivalents (FGEs), or the number of unrelated founder parents that would represent the same amount of GD as observed in the current population, was also initially modest (approximately 55-60) in the years 2000-2003, but it then increased through the period 2004-2009 to approximately 75-85, before slowly declining to the low 1960s by the end of the

study period. When assessed by generation, however, FGE was slightly higher (~100) and did not appear do decline from G0 to G2 sets of Salmon spawned.

The number of parent Founder Alleles (FA) retained varied greatly from year to year. In 2000-2003, values ranged from approximately 260 in 2002, to over 300 in 2000. The highest yearly value was seen in 2004 (nearly 380), then levels observed in every successive year were generally lower than the previous year thorough to 2014, where FA was approximately 175, less than half of that observed in 2004. When tabulated by approximate generation, levels of FA were markedly higher but it also declined over time, from well over 600 in the G0 to just over 400 in the G2.

Expected molecular genetic variation increased through the first 5 years, declined slightly year-over-year thereafter, but changed little across the duration of the period assessed (2000 to 2015). Observed molecular genetic variation generally appeared to have increased slightly between 2000 and 2013, but it may have declined at a moderate rate thereafter (Figure 9). The timing of the decline coincides with a marked increase in the observed co-occurrence of G1 HS common ancestors between pairs of Salmon spawned in 2010 and 2011 (between parent sets that produced the 2014 and 2015 Salmon that exhibit possible reductions in observed heterozygosity). In other words, some of this reduction in observed heterozygosity may be due to increases in inbreeding resulting from the original bottleneck, which is expected to occur at this time. The number of observed molecular genetic marker alleles (another metric of levels of genetic variation, not to be confused with G1 parent FA discussed above) decreased slightly from 2000 to 2015 (Figure 10); nearly all alleles lost were either putative short European variants directly or indirectly selected against, or alleles initially present at very low frequencies (typically, 1 in approximately 2000 initial FA assayed). Overall, rates of loss of molecular genetic variation between the STW G0 (founder) and second generation (G2) Salmon were low, though some slight reductions were observed.

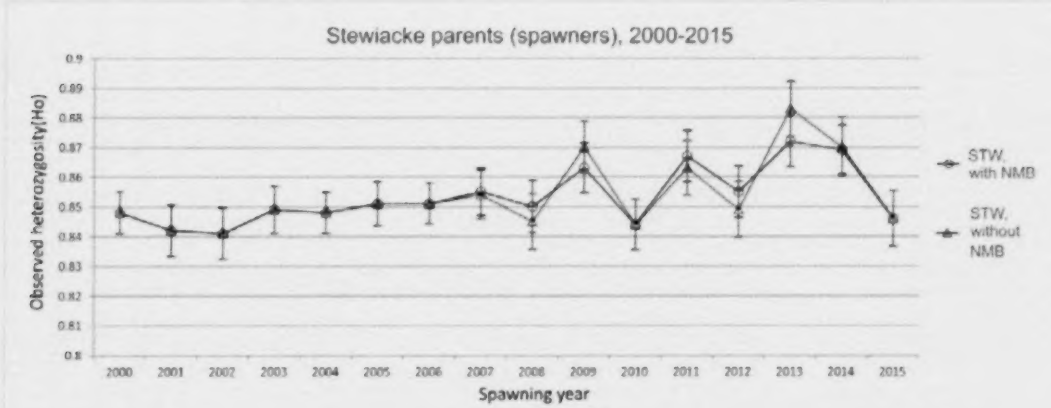


Figure 9. Observed heterozygosity (Ho) of Stewiacke River LGB parents (spawners) in each of the years 2000-2015, with and without non-Stewiacke NMB founders and their ancestors. Standard errors are also given. Results based on 8 microsatellite loci.

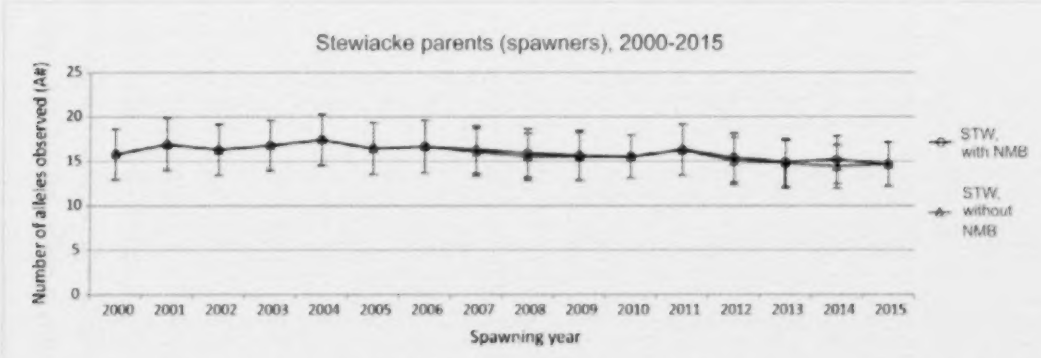


Figure 10. Number of alleles observed (A#) in Stewiacke River LGB parents (spawners) in each of the years 2000-2015, with and without non-Stewiacke NMB founders and their ancestors. Standard errors are also given. Results based on 8 microsatellite loci.

Drift-induced Changes in Overall Allele Frequency Distributions Through Time

Allele frequency distributions of large groups of STW Atlantic Salmon spawned in early program years (2000-2003) were often significantly different from allele frequency distributions of similarly large groups of Salmon spawned in the latest years included in this study (2012-2015). Additionally, pairwise estimates of differentiation (FST) between these same early and later spawner groups were often approximately 0.002 and as high as 0.004. However, much of the divergence between these groups is likely due to differences in family compositions of wild parr samples obtained in each of the years 1998 to 2001, varying distributions of these four groups in sets of Salmon spawned in 2000 to 2003, and homogenization of the population (with respect to the original family lineages) in subsequent sets of spawned Salmon. In other words, drift-induced changes in overall allele frequency distributions between STW G0 and G2 generation Salmon have been very modest (FST~0.001 or less). Once among-year differences in founder collections are accounted for, no significant differences among yearly spawner groups across the duration of the program were observed.

Effective Number of Breeders, Effective Population Size, and Expected Rates of Loss of Genetic Variation

The effective number of breeders associated with STW River offspring produced in a given year was generally quite high, ranging from 200 to approximately 400. The effective population size (Ne), based on tabulations of individual life-time reproductive success, was often lower, ranging from approximately 100 to 300, and increased in later years. The generational effective population size was likely higher (above 500), and it increased in later years.

Given STW River Atlantic Salmon population demographics observed in recent years, and the discrete and non-overlapping nature of the STW population from 2013 on (see O'Reilly et al. 2018), the wild-exposed subpopulations managed as recommended are expected to retain greater than 95% of GD across 20 Salmon generations; GD when assessed across the entire population will be even higher. These 5 sub-populations are expected to accumulate inbreeding equal to F=0.03 to 0.04 across 20 Salmon generations; however, this can be reduced by permitting limited ongoing gene flow between year classes in the future.

If RMK breeding protocols are followed in the future, rates of loss of GD and accumulation of inbreeding are expected to be even lower than that indicated above, which was solely based on effective population size.

Variance in Family Size and Effective Number of Breeders Observed in Non-selected Captive-reared Versus Wild-exposed Offspring Groups for the Spawning Years 2002-2010

In the STW iBoF LGB program, there are two offspring group types, (1) those reared exclusively in captivity (captive-reared) and (2) those initially reared in captivity, subsequently released into the wild for 1-2 years of exposure to native river conditions and natural selection, and then recaptured for additional captive rearing through to spawning (wild-exposed). Variance in family size, effective number of breeders in the parent generation, and expected rates of loss of genetic variation (assuming non-overlapping population characteristics and constant population size) were estimated for multiple brood years based on these two offspring group types. Overall, variance in family size was lower for captive-reared versus wild-exposed groups from a given brood year. All else being equal, rates of loss of genetic variation, in nearly all within-brood year comparisons, would be higher for comparable wild-exposed offspring groups. However, from 2006 on, when important changes were made to the management of wild-exposed offspring groups (e.g., equalization and release into the Pembroke River), differences in the magnitude of variance in family size between captive-reared and wild-exposed offspring groups in any within-brood year comparison, was markedly reduced. Expected rates of loss of genetic variation under a management regime consisting of exclusively wild-exposed offspring would be only slightly elevated relative to a management regime consisting of exclusively captive-reared Salmon, and a regime consisting of captive-reared and wild-exposed offspring groups. However, estimated effective number of STW breeders (which from 2013 on will be equivalent to effective population size), under the wild-exposed offspring management regime, is probably sufficiently large that rates of loss of genetic variation due to random genetic drift going forward, and rates of accumulation of inbreeding, are not expected to be high.

Effective Population Size, Gene Diversity and Accumulation of Inbreeding Expected After 20 Generations under Different Management Regimes Available for inner Bay of Fundy Atlantic Salmon

There are several possible pathways to maintaining large effective population size and achieving program goals (O'Reilly et al. 2018). Each of these different pathways involves different levels of expenditures of three key resources: genotyping capacity (allowing pedigree-placement of individuals); hatchery space; and human resource capacity (year-round associated with adult census population size and intensive spring-fall associated with field operations required for the release and recapturing wild-exposed offspring). Actual values of variance in family size for captive-reared, wild-exposed, and captive-reared + wild-offspring groups, before and after the application of pedigree information, were used to estimate N_e, GD, and level of inbreeding (F) at G1, G10 and G20 generations under different management scenarios (assuming populations were discrete and non-overlapping, as is the case for the STW LGB from 2013 on).

A goal of retaining 95% of GD through to the G20 generation could be achieved by, for example, (a) maintaining the adult census population at 150 (carrying out 75 paired spawnings), rearing juveniles in captive and wild environments, and using genotype information to further minimize variance in family size at spawning time, (b) increasing the adult census population to 250 (carrying out 125 paired spawnings), rearing juveniles in exclusively captive environments, but without pedigree information, or (c) increasing the adult census population size to 300 (carrying out 150 paired spawnings) and rearing juveniles in the wild, without pedigree information (but after equalizing family size). Note that increasing the adult census population size requires carrying out more crosses, rearing and managing more egg lots, and rearing more offspring through to release and to spawning. The optimal strategy chosen by management, given the sole consideration of conserving genetic variation, would depend on the availability of

various resources, including hatchery space, human resource capacity, and genotyping capacity. The main caveat with these results is that they are based on estimates of variance in family size carried out in 2002-2010; high selection intensities in future years in either hatchery or wild environments, and/or large random family-specific mortality across a handful of egg trays/baskets could markedly increase variance in family size in some years relative to earlier years when estimates used here were made. Variance in family size, N_e, and expected rates of loss of genetic variation would need to be monitored. In other words, some genotyping of a reasonable portion of the parents and associated offspring in some years is recommended.

Expected Efficacy of Different Founder Selection Regimes in Minimizing Loss of Genetic Variation and Inbreeding in Bottlenecked Populations of Atlantic Salmon

Given accelerating declines in population abundance of Atlantic Salmon in neighboring Maritimes DUs, and the possible need for similar captive breeding and rearing programs in the future, the expected efficiency of different founder selection regimes in minimizing rates of loss of genetic variation was investigated. First, modelling-based analyses were used to test the five different methods, Minimization of Mean Kinship (MMK), Mean Kinship Assist (MKA), Ranked Mean Kinship (RMK), Minimizing Pairwise Relatedness (MPR), and Random Selection and Pairing (RSM), of selecting variably sized subsets (50-150 individuals) of founders from the large collections of approximately 1000 iBoF wild parr obtained in 1998 to 2001. Generally speaking (when selecting 50, 100 or 150 founders), MPR captured more molecular genetic variation than all other methods, but this likely reflects the direct role that the marker data used has in both selecting founders and in tabulations of levels of molecular diversity in the subgroups selected. Additionally, HS and FS family diversity was lowest under MPR and is, therefore, not recommended. Levels of molecular genetic diversity were next highest in sets of 50 and 100 founders selected using MMK, but when the number of founders was increased to 150, RMK performed best. Levels of FS and HS family diversity were generally highest under MKA and RMK, regardless of the size of the founder group selected. Overall, these results collectively indicate that genetic diversity may be highest, at least initially, in moderate to large founder groups obtained from larger collections of G0 Salmon using RMK methods.

Additional modelling analyses, carried out using the program PMx (Lacy et al. 2012), were performed to test the efficacy of six different founder selection regimes, Maximum G1 HS Founder Evenness Regime (MHFER), Maximum G1 FS Founder Representation Regime (MFFRR), Intermediate Founder Selection Regime 1 (IFSR1), Intermediate Founder Selection Regime 2 (IFSR2), Ranked MK 1 (RMK1) and Ranked MK 2 (RMK2), in minimizing expected rates of loss of genetic variation (and accumulation of inbreeding) across 10 generations of captive breeding and rearing. Analyses were based on the original set of 401 parr obtained from the STW River in 1998, with one small simulated HS family of 10 individuals included in order to facilitate analyses. Neither MHFER nor MFFRR is likely to result in the lowest rate of loss of genetic variation (or accumulation of inbreeding) across 10 future generations of captive breeding and rearing. Instead, a spawner selection regime using Ranked Mean Kinship (RMK2) to identify a subset of G0 Salmon as founders lead to, overall, the lowest rates of loss of GD, FA, and accumulation of inbreeding over time, though results were somewhat dependent on the number of founders selected from the larger G0 group.

Introgression of Non-native (Non inner Bay of Fundy) Genes into the Big Salmon River Live Gene Bank Gene Pool

Given (1) population demographics of SJR and iBoF Salmon in recent years, (2) expected changing proportions of SJR first generation adult stays to native returns in the BSR adults over time, and (3) the collection and use of 'in river' produced smolt in the BSR LGB program,

increasing introgression of non-local genes into the BSR gene pool over time could be expected.

Genetic structuring between outer Bay of Fundy (oBoF) and BSR Salmon appears to be decreasing over time. Results from several population genetic analyses are all consistent with extensive and ongoing gene flow from non-local (likely oBoF) sources into the BSR gene pool, and this could represent a risk to the conservation of iBoF genetic characteristics in the BSR LGB population.

Based on information from multiple nuclear and mitochondrial DNA markers, European farm Salmon ancestry has been detected in Salmon collected from the Upper Salmon River (USR), with a very high degree of certainty. Information from multiple nuclear markers indicates that European farm ancestry has also been detected in additional iBoF rivers (BSR, GAK and STW), with a high degree of certainty. Additional analyses involving multiple marker loci indicate that European farm Salmon (including likely wholly European Salmon) appear to have successfully reproduced in several iBoF rivers (USR, BSR, GAK and STW), and in many instances, appear to have spawned with endangered iBoF Atlantic Salmon.

If short European-type *Ssa*202 variants alone reflect the presence of European farm ancestry (O'Reilly et al. 2018), the spatial and temporal scope of influence of European farm Salmon escapes in the iBoF may be even larger. The frequency of detection of short European-type *Ssa*202 alleles in sample collections of Atlantic Salmon (primarily juveniles) obtained from rivers of the Bay of Fundy area increased markedly in and around 1998-2000, just a few years after European farm Salmon genetic material was introduced into Maine, and immediately following expected increases in the prevalence of European ancestry in net pen reared Salmon in the Bay of Fundy/Gulf of Maine area (Figure 11).

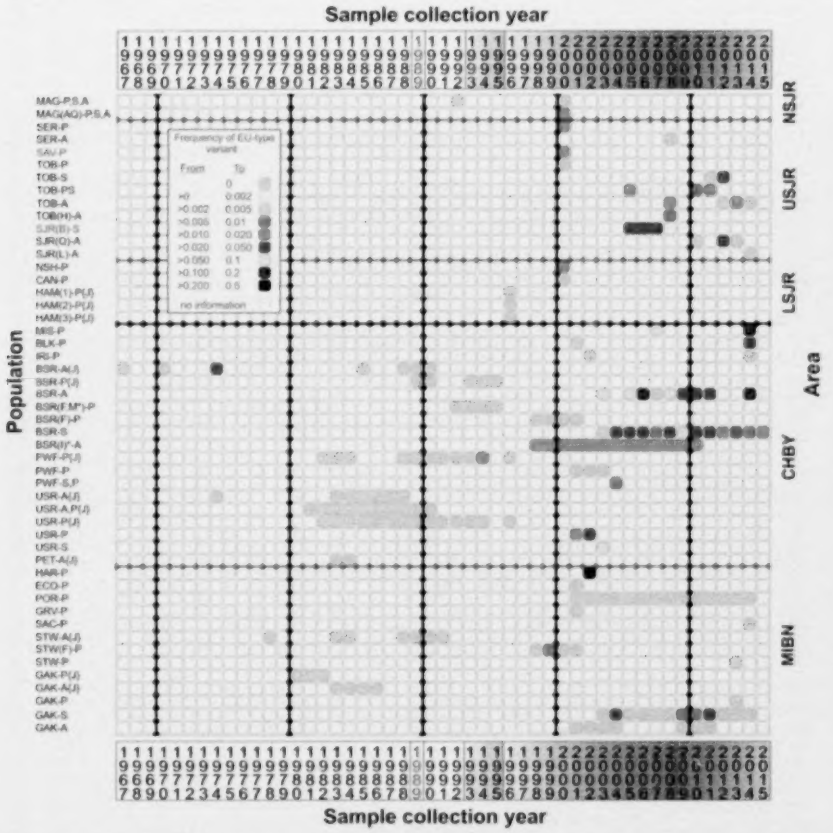


Figure 11. Frequencies of short European-type Ssa202 alleles (<255 bp) in sample collections of Atlantic Salmon obtained from rivers of the Bay of Fundy (first three characters, Y-axis, left side of figure) in the years 1967-2015 (X-axis). Each polygon represents a sample collection obtained in a given year from a particular river, with colour denoting the frequency of short European type Ssa202 alleles; white indicates the absence of any sample information, grey indicates that short European type Ssa202 alleles were not observed. Sub-region or area is indicated on the Y-axis on the right (NSJR-non Saint John River; USJR-Upper Saint John River; LSJR-Lower Saint John River, CHBY-Chignecto Bay side of the inner, MIBN-Minas Basin side of the inner Bay). European farm Salmon were first imported into the area in 1989, but offspring of escapes (Age 0 fry, Age 1 parr and Age 2 smolt) would not be expected to be sampled in nearby rivers until 1993-1995 at the very earliest, with detections more likely from 1996 on (see X-axis). See Table AII6 in O'Reilly et al. (2018) for river names associated three letter identifiers, and other details regarding characteristics of sample collections including sample size.

These and other results in O'Reilly et al. (2018) indicate that local (Bay of Fundy/Gulf of Maine) farm Salmon escapes exhibiting European ancestry, or their European farm/North American farm hybrid offspring, appear to have been spawning in the iBoF during the period spanning 1997 to 2012, including during all or most years within this period; this could have continued in later years but cannot be addressed without additional samples. These results may also indicate that European farm Salmon, or their European farm/North American hybrid offspring, have spawned in multiple iBoF rivers (nearly all sampled) (Figure 11).

Based on analyses of Ssa202, European farm/North American hybrid Salmon (N=1 to 4) have been detected, with a moderate degree of certainty, in almost all yearly sample collections of adult Atlantic Salmon (N=7 to 34, Mean=15.3) obtained from the BSR, including those from

2003, 2005, 2006, 2008, 2010, 2011 and 2014; the 2007 collection of 8 individuals did not contain any detectable European farm/North American hybrids, and no samples were collected (and analyzed) from years 2004, 2012 and 2013. Multiple (dozens) of European farm/North American hybrid Salmon, possibly 2 to 3 generations removed from the original pure European parents, have been detected, with a moderate degree of certainty, in all yearly collections of 'in river' produced smolt obtained on the BSR from 2004 to present; these individuals are collected for spawning in the BSR LGB program. Given the observed detection rate of short Ssa202 alleles in BSR smolt, and expected reductions in frequencies of these alleles expected in the offspring of hybrid-North America backcrosses, a fairly large proportion (approximately 10 to 25%) of 'in river' produced BSR smolt may exhibit some level of European farm ancestry.

The spatial and temporal occurrence (and frequency) of spawning of European farm Salmon appears to be higher in Chignecto Bay compared to Minas Basin rivers (Figure 11), perhaps related to the coastal distance between the mouths of these rivers and Passamaquoddy/ Cobscook bays (sites of intensive Salmon farming), and movement/survival of farm Salmon escapes (see Figure 1).

Both male and female European farm escapes appear to be successfully reproducing in rivers of the iBoF. Suspect F1 to F4 European farm/North American hybrids, also inferred to have spawned in inner bay rivers, are most often FS male parents, probably mature male parr. These findings have implications for both the initial rate of introgression of European farm genes into wild populations, but also for the persistence of European farm genes across future generations.

One BSR adult (BSR140905SOAD21) exhibiting an European-type Ssa202 allele produced 6 returning adults, and another 2 adults also exhibiting an European-type Ssa202 allele (BSR070906SOAD0619 and BSR140905SOAD19) produced one returning adult. Both BSR140905SOAD21 and BSR070906SOAD0619 assigned to a common HS group, though they appear to have inherited their Ssa202-247 allele from different male parents. The lineage of Salmon associated with BSR140905SOAD21 persisted across a minimum of four generations, two of which included individuals that went to sea and returned, an interesting observation given exceedingly low average adult-to-adult lifetime reproductive success in the iBoF for this species at this time.

The F3 to F4 European farm/North American hybrids may exhibit reduced early juvenile survival relative to pure STW x STW parents, in wild STW River habitat.

European farm/North American hybrids were present in the original early STW and BSR (1998-2001) and GAK (2001-2004) founder collections but were probably very rare. Nevertheless, descendants of the original European farm/North American hybrids were present in the 2016 candidate spawners and will also be encountered in 2017-2020 candidate spawners.

Additional laboratory studies (including analyses of the microsatellite variation at the locus Ss1) are recommended to further test hypotheses of European ancestry in BSR Salmon, especially those exhibiting a single short European-type allele.

Using pedigree information, most or all Atlantic Salmon exhibiting European farm ancestry can probably be removed from the STW LGB population with minimal risk; additional analyses need to be carried out to determine the relative costs (increased loss of native genetic variation) and benefits (reduced prevalence of highly divergent genetic material) of attempting to removing European farm ancestry from the GAK LGB population. Removing European farm ancestry from the BSR LGB population may not be possible using conventional methodologies.

Effects of Alternate Management Regimes on the Several Measures of Offspring Performance (Including Survival) in Captivity and in the Wild

There was no evidence for possible effects of parental early juvenile rearing environment (captive versus wild) on early offspring survival (release to Age 1, 16 months post-release) in the wild. In some years, the parental early juvenile rearing environment (captive versus wild) may have influenced size-at-age of offspring in their first year in the wild, with offspring of wild-exposed Salmon exhibiting greater length and weight compared to offspring of exclusively captive-reared parents. No evidence was found for possible cumulative negative effects of ancestral (parent and grandparent) early juvenile captive rearing environments on early offspring survival (release to Age 1, 16 months post-release) in the wild.

In some years, a possible negative influence of increasing number of generations of captive breeding and rearing on survival in the wild was observed and, although the effect was small, the number of generations across which comparisons were possible (1-1.5) was very limited.

There was no evidence for possible effects of mean standardized parent family size in the wild (a proxy for parent family survival or fitness) on offspring family survival in the wild; these results minimize concerns over preferentially spawning low MK Salmon, when MK values partially reflect family size in the wild.

In one of four years assessed (2010), pedigree-based MK was strongly positively correlated with family survival in the wild; this is a potential concern because RMK spawning algorithms prioritize low MK individuals for spawning, potentially selecting for lower survival in the wild. However, in 2010, survival in the wild was also negatively correlated with number of program generations, with individuals exhibiting more program generations tending to exhibit lower survival. Mean Kinship and the number of program generations are themselves likely to be correlated, because relatedness is expected to increase over time (across generations). This apparent association between MK and survival in this one year may actually reflect the effect of increasing program generations on offspring survival. In addition to these results,

- the lack of any association between MK and percent survival in other years,
- the absence of any direct association between parent family size in the wild and offspring family size in the wild, and
- the available literature on other species showing no relationship between MK and other indicators of fitness,

suggest that this apparent association is indeed a likely artefact. However, it is recommended that survival in the wild continue to be monitored in the context of MK in the future.

No relationship between pedigree inbreeding and either offspring performance (survival or size) in the wild, or the occurrence or percent deformities of fry in captivity, were observed. No statistically significant relationship was observed between expected observed molecular genetic heterozygosity in the offspring, and family survival in the wild from release to Age 1.

Offspring of outbred crosses (STW x ECO, STW x GRV or STW x DEB) appear to have exhibited higher survival in the wild from release to Age 1 compared to offspring of non-outbred crosses (STW x STW) in the one year where data were available. Offspring of outbred crosses (STW x GAK) did not appear to exhibit higher shock-to-release survival in captivity than did offspring of non-outbred crosses (STW x STW or GAK x GAK). Offspring of outbred crosses (STW x GAK) did not appear to exhibit lower percentage of fry deformities compared to offspring of non-outbred crosses (STW x STW or GAK x GAK). Release to Age 1 survival results (in the wild) for STW x STW, STW x GAK and GAK x GAK) crosses will be available in

the fall of 2017 and will further inform decisions regarding whether or not to introgress GAK genes into the STW population.

No association between measures of molecular genetic variation or pedigree inbreeding and either survival in the wild or the incidence or percentage of fry deformities was observed, though offspring of hybrid crosses between Economy or Great Village and STW.

Female parent age, but not male parent age, was found to have a large effect on multiple offspring performance traits, including survival in the wild from release to Age 1, and this effect was consistent across multiple years; part of this effect appears to be due to egg size, but female parent age was also associated with increased survival independent of egg size in some years.

Generally, most monitored traits in the STW population did not show any discernible trends over the course of the program, with a few exceptions. Survival from 'fertilization to shock' appears to have increased over time. However, this apparent trend may actually reflect increasing fertilization rates or fertilization success across years. Many dead eggs were initially identified as post-fertilization mortalities. However, additional analyses indicate that most were likely never successfully fertilized. After correcting for fertilization rates, survival from fertilization to shock was likely quite high (above 99%) and remained high (above 96%) from shock to pre-release across all years assessed.

Fry deformities, although already occurring at a very low rate of <1%, appear to have decreased over the last 6 years of the LGB program.

More Age 2 females leave as smolts compared to males, which likely remain in the river to mature as precious parr.

Captive-reared STW spawning adults are larger than wild-exposed and wild-origin individuals; they are maturing at an earlier age than their wild-exposed counterparts and the percent of captive-reared adults maturing at Age 4 is increasing over the years. There was no difference in length between wild-exposed and wild-origin adults at spawning (Age 4) suggesting that the naturalization of the rearing environment may be mitigating some program effects on this trait.

Status of iBoF Atlantic Salmon and the Different Release Strategies of the LGB Program

Atlantic Salmon monitoring activities, undertaken from 2001 to 2016, associated with the LGB program have included and/or continue to include: two updated electrofishing surveys for juvenile Salmon (2013 and 2014), annual mark-recapture estimates of numbers of smolts emigrating from the BSR and GAK rivers, annual mark-recapture estimate of adults returning to the BSR River, annual counts of adult Salmon returning to the GAK River, a summary of annual LGB collections and distribution, and an assessment of a crossbreeding experiment in the Pollet River, a tributary of the Petitcodiac River (Jones et al. 2018). All of the assessment activities incorporate genetic analyses to evaluate the success of the LGB in preventing the extirpation of this endangered Salmon population.

Electrofishing Surveys

Stewiacke (2013)

An extensive electrofishing survey was conducted between August 20th and September 11, 2013, throughout the STW River. The goals of the survey were: to calculate the Salmon densities in the various tributaries, to determine if wild spawning is occurring, and to investigate the contribution of LGB spawners (i.e., as returning adults) to the Salmon population within the STW River. A total of 40 sites were surveyed of which 11 were historical reference sites

previously sampled for monitoring trends in juvenile abundance. A total of 234 fry and 168 parr were collected and tissue sampled, and 379 juveniles were transferred to the Coldbrook Biodiversity Facility. Parentage analysis assigned 345 juveniles to parent pairs or single parents, leaving 48 juveniles with one or both parents undetermined. A grand-parentage analysis was then conducted, after two additional loci were added, which allowed for the assignment of two sets of grandparents to 34 of the 48 juveniles, one set of grandparents to a further 13 juveniles, and none to only one juvenile. These results indicate the presence of two 'unknown' female spawners that could be suspected wild spawners or could also be from non-genotyped Salmon in the system.

Broadscale (2014)

In order to evaluate recent status of juvenile abundance in non-supported iBoF rivers, an extensive electrofishing survey involving many collaborators was completed in 2014. A total of 34 rivers were included in the project in which 85 sites were surveyed between August 11 and October 2, 2014. Salmon presence was only detected in seven of the 34 rivers surveyed and at low densities: Mispec, Mosher, Black and Irish rivers in New Brunswick and Salmon (Truro), Portapique and Great Village rivers in Nova Scotia. Genetic analysis on the Salmon caught in the New Brunswick rivers indicated that those fish exhibited a predominantly oBoF baseline profile based on the likelihood model suggesting progeny of possible strays. The only LGB supported river in the survey, the Salmon River (Truro), was surveyed to evaluate the spawning success of the non-targeted adults released into this tributary, as there was very little evidence that previous adult releases had successfully spawned. Salmon were detected in all 3 sites surveyed, and 4 parr, 39 parr and 2 fry were captured. Parentage analysis assigned 40 of 45 individuals to wild spawning adults released from the STW LGB program leaving 5 juveniles with one or both parents undetermined.

The observation that juvenile Salmon are no longer present, or if present are at extremely low densities in the 33 non-LGB supported rivers sampled during an extensive broad scale electrofishing survey, indicates the lack of returning truly wild iBoF Salmon and that iBoF Salmon would likely be extirpated without the LGB program (Figure 12).

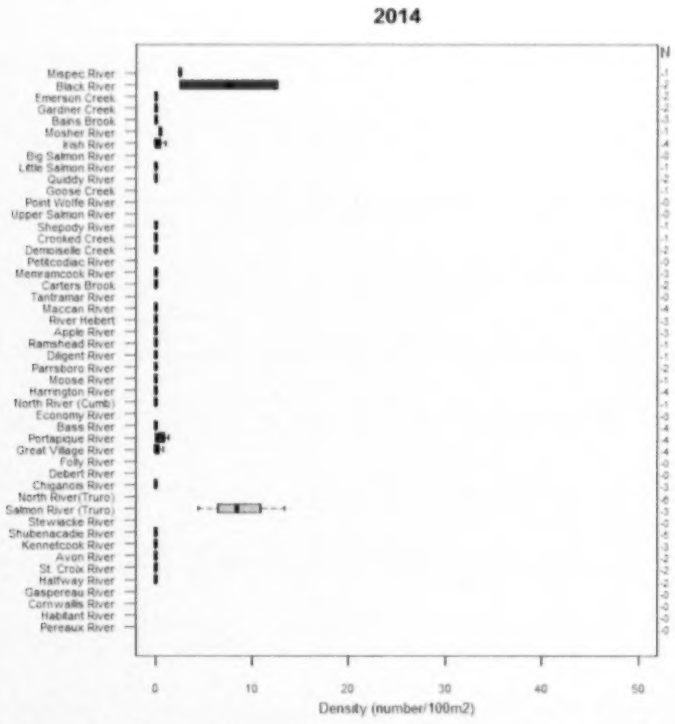


Figure 12. Densities of juvenile Atlantic Salmon in inner Bay of Fundy rivers based on electrofishing surveys completed in 2014. The column "N" represents the number of sites electrofished and black rectangle represents the median density. The whiskers depict the minimum and maximum densities observed each year. The LGB-supported rivers are in orange and non-supported in blue. Rivers with blank spaces were not electrofished.

Smolt Assessment

To facilitate the annual collection of smolts for the LGB program, annual smolt assessment programs were initiated on the BSR and GAK rivers to estimate smolt abundance by origin. This smolt monitoring data is used to assess in-river and marine survival for the progeny of the LGB program and any remnant wild adult spawners. Whenever possible, mark-recapture techniques are used to determine the smolt abundance estimates on these rivers. Smolt and parr releases from the LGB program were easily discernable from wild or LGB fry releases by the absence of the adipose fin. The origin (from adult spawners or LGB unfed fry) of the non-adipose clipped smolts, however, could only be determined through the use of genetics. Genetic analysis (or parentage assignment) of tissue samples randomly collected from outgoing non-adipose clipped smolts, in combination with assessment data, provides smolt abundance estimates by origin. The non-adipose clipped smolts that did not assign to the parents of the LGB program are grouped as 'adult spawners' and would include progeny from the remnant wild population.

Since 2001, emigrating smolts have been captured on the BSR River using a RST that is annually installed near the mouth of the river at the upstream end of Amateur Pool from early May until mid-June. On the GAK River, downstream fish passage is provided for smolts via three surface bypass structures that contain assessment traps which are typically monitored (smolts enumerated) between mid-April until the end of May, although time period are adjusted to the smolt 'emigration window' based on water temperatures and seasonal flows.

On the BSR River, the mean annual smolt production from 2003 until 2016 has been about 13,400 smolts. Over this time period, the annual contributions by origin breakdown as follows: adult spawners have contributed 4,729 fish, LGB unfed fry releases have contributed 4,646 fish and adipose-clipped LGB fall and spring parr releases have produced 4,034 fish. The biological characteristics and in-river survival of the two main release strategies (i.e., LGB fry and LGB parr) continue to differ over the time period. The 'in river' survival of LGB parr from the time of release to the smolt stage is about 3 times greater than that of the LGB unfed fry releases (6.6% versus 1.8%) (Figure 13), although smolts that result from the unfed fry releases are more comparable biologically (i.e., run timing, size at age, age distribution) to the smolts produced from the adult spawners (see Jones et al. 2018 for more details).

Since 2007, the total number of smolts emigrating from upriver of White Rock Dam on the Gaspereau River has ranged between 1,174 and 7,354 fish and averaging about 4,200 smolts annually.

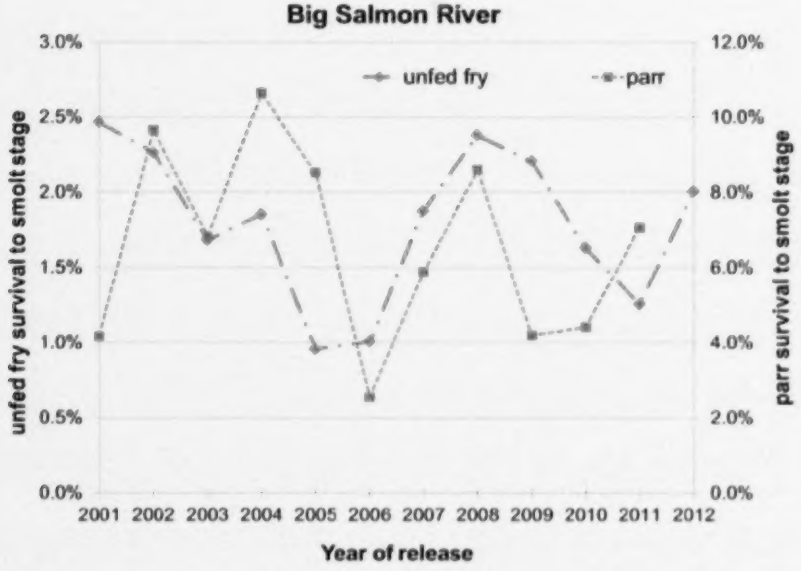


Figure 13. Live Gene Bank unfed fry and parr releases and percent survival to smolt stage on the Big Salmon River.

Adult Assessment

The two index rivers monitored for adult abundance within the iBoF DU, and include LGB programs, are the BSR (New Brunswick) and the GAK (Nova Scotia) rivers. Status is evaluated using a comparison of the estimated egg deposition (calculated from the estimated abundance and biological characteristics of Salmon stocks) relative to a reference point known as the conservation egg requirement. The river-specific conservation egg requirement is based on an egg deposition of 2.4 eggs/m^2 multiplied by the amount of accessible fluvial rearing habitat that is of suitable gradient.

Adult abundance estimates on the BSR River have been ongoing annually since 2001 using the following approach: an early season diver count of Salmon holding in the largest pools in August, a mid-season count usually in September of those same pools followed by a seining/marking activity, and finally a three-section swim survey in October. If sufficient numbers

of Salmon were tagged, an abundance estimate was generated, if not, a single census mark-recapture value (0.57) was applied to the count.

Adult returns to the GAK River are monitored by counting the small and large Salmon captured in a pool and weir fishway designed to bypass the White Rock Dam. Salmon have been enumerated at the White Rock Dam since 1995, and following retrofitting in 2002 to include an assessment trap, transported and held at the Coldbrook Biodiversity Facility for possible inclusion into the LGB program.

Since the LGB program was initiated on the BSR and GAK rivers, most of the adult returns captured on either river have been tissue sampled to determine origin of returns. Based the genetic analysis, about 24% of the small Salmon returns on the BSR River (Figure 14) and the majority (71% of small and 73% of large Salmon) of the adult returns to White Rock Dam on the GAK River have been the direct result of the LGB program (Figure 15).

Both GAK and BSR rivers remain well below conservation egg requirement due to poor marine survival. The smolt to small Salmon return rate from the combined group of adult spawners, LGB fry, and LGB parr has been consistently below 1% and has averaged 0.32% from 2002 to 2016 on the BSR (Figure 16).

The mean smolt to small Salmon return rate for the smolts that originated from LGB fry is 0.18% which is about 3 times better than the mean return rate for LGB origin smolts released as parr (Figure 16, Table 1).

The smolt to adult return rates observed on the GAK River since 2007 are also very low but could also be affected by upstream passage issues at White Rock Dam. Other habitat connectivity/fish passage issues were observed during the large scale electrofishing survey, which likely affects the presence of Salmon in several iBoF rivers (Jones et al. 2018).

A summary of the comparative biological characteristics in terms of; size distribution, age distribution, and sex ratio between 'wild' (adult spawners) and LGB origin adult returns to the BSR and GAK rivers, indicated similar results between the groups on both rivers with the exception of a lower proportion of females in the LGB origin 1SW Salmon on the GAK River (Jones et al. 2018).

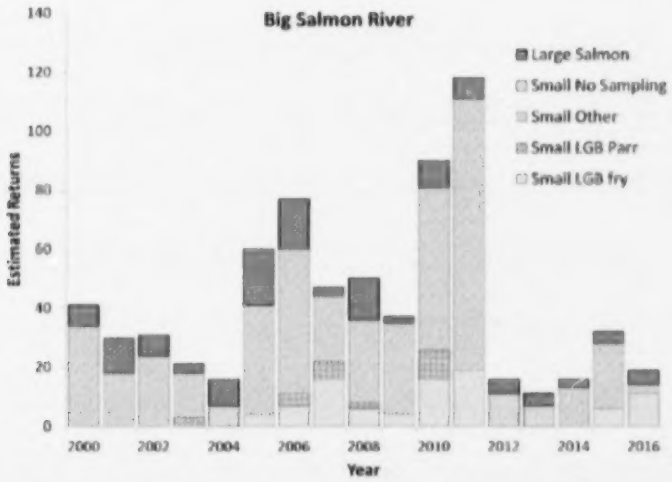


Figure 14. Estimated small and large Salmon returns to the Big Salmon River, 2000 – 2016. Small Other = progeny of previous adult spawners or small Salmon of unknown origin.

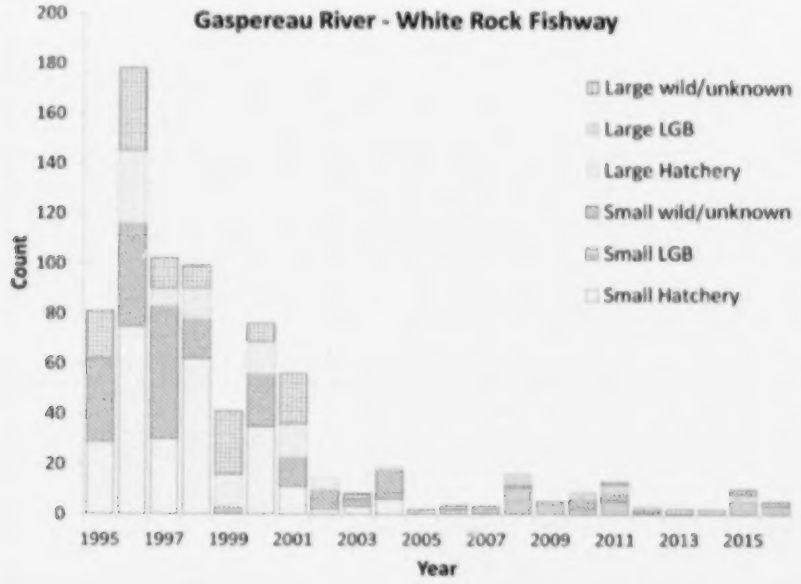


Figure 15. Small and large Salmon counts to the fishway at the White Rock Dam on the Gaspereau River, from 1995 to 2016.

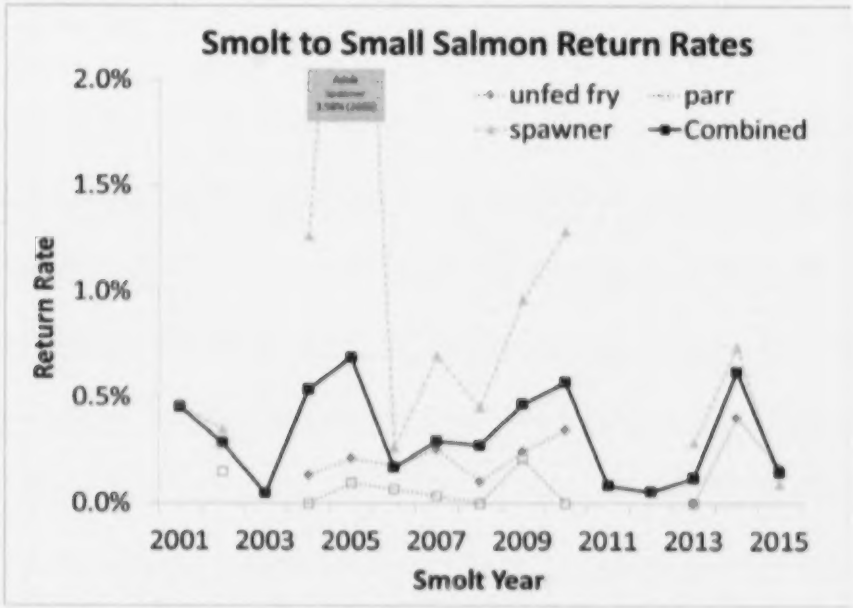


Figure 16. Smolt to small Salmon return rates from the Big Salmon River.

Table 1. Estimated smolt-to-small Salmon return rates for smolts of LGB origin (fry and parr releases) and those from adult spawners on the Big Salmon River, 2002–2016. N/A=Not applicable.

		Combined[1]			Smolt-to-small Salmon Return Rate by Origin			
Smolt Year	LGB Smolt	LGB Unfed Fry	LGB Parr	Adult Spawners	LGB Unfed Fry	LGB Parr	Adult Spawner	Combined[1]
2001	0	N/A	N/A	5,290	N/A	N/A	0.45%	0.45%
2002	19,725	N/A	2,035	4,295	N/A	0.15%	0.35%	0.28%
2003	13,647	3,640	6,120	5,560	No data	No data	No data	0.05%
2004	13,224	3,036	1,691	2,934	0.13%	0.00%	1.26%	0.54%
2005	1,296	3,320	4,175	1,230	0.21%	0.10%	3.98%	0.69%
2006	1,413	8,954	8,940	8,401	0.18%	0.07%	0.26%	0.17%
2007	0	2,363	5,855	4,037	0.25%	0.03%	0.69%	0.29%
2008	0	3,909	2,110	6,841	0.10%	0.00%	0.45%	0.27%
2009	2,072	6,568	4,756	5,392	0.24%	0.21%	0.96%	0.47%
2010	2,077	5,464	6,840	7,156	0.35%	0.00%	1.29%	0.57%
2011	432	4,543	2,939	5,592	No data	No data	No data	0.08%
2012	0	4,239	1,900	6,881	No data	No data	No data	0.05%
2013	0	5,350	1,050	4,490	0.00%	0.00%	0.29%	0.12%
2014	0	1,482	40	2,988	0.40%	0.00%	0.74%	0.62%
2015	0	6,435	0	3,255	0.17%	N/A	0.09%	0.14%

[1] Combined values excludes LGB smolt releases.

Crossbreeding Experiment in the Petitcodiac River

The Petitcodiac River is thought to have produced 20% of the iBoF population prior to the causeway being built in the late 1960s. The combination of the construction of the causeway with ineffective upstream and downstream fish passage and high marine mortality effectively extirpated the Salmon population from this large watershed. The opening of the Petitcodiac River causeway gates in 2010 removed the fish passage problem in the system. This pristine habitat presented an opportunity to conduct multiple crosses among the various iBoF populations (i.e., Stewiacke, Gaspereau, New Minas Basin, Point Wolfe and Big Salmon rivers) housed within the DFO Biodiversity Facilities to determine which or if a unique cross or specific river cross might produce smolts better suited to this system as exhibit improved marine survival. As a result of the experiment, 337,622 and 37,246 unfed fry were released into the Pollet River in 2011 and 2012, respectively.

Fort Folly Habitat Recovery has evaluated the survival of the fry releases from various crosses with the tissue sampling of smolt captured in fyke nets and a RST operated on the Pollet River during the spring of 2013, 2014 and 2015. Mark and recapture techniques were used to estimate smolt abundance in those years. In 2014, 2015, and 2016, adult surveys were conducted in late-September, a three-section swim survey was conducted in the Pollet River to note the presence of small Salmon returning from the fry releases but very few adults were observed in all three years (Jones et al. 2018).

The results of the cross-breeding experiment in the Pollet River indicated that the unfed fry releases in 2011 from the BSR River crosses survived about 2.3 times better to the smolt stage than the unfed fry released from the combined NS LGB program outcrosses. Interestingly, despite being reared in similar environmental conditions, the growth rates of the Age 2 smolts appeared to be influenced by their 'genetic' river of origin (Figure 17). The progeny from the STW x STW parents were the smallest and the smolts that originated from GAK x GAK river

parents origin smolts were the largest while the BSR x BSR smolts were an intermediate size that generally corresponds with the river specific data collected from 2014 to 2016.

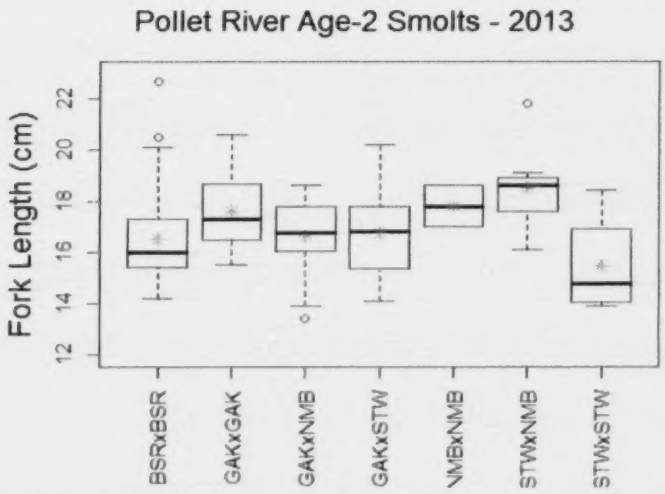


Figure 17. Summary of fork length data by origin (cross) for Age 2 LGB smolts sampled during assessment projects on the Pollet River in 2013. Plots show the median and the 25th and 75th percentiles. Error bars represent the 10th and 90th percentiles, with outliers denoted as circles. The red asterisk represents the mean.

Sources of Uncertainty

- Funding limitations in some years, and operational constraints (changing genotyping platforms and inability to assay length variation at some loci) in others, has resulted in changes in the set of loci analysed in groups of Salmon spawned in a given year over the duration of the program. As a result, some population estimates of genetic diversity, especially those involving full length time series (15 years), involve fewer loci, and will be less precise.
- When FS and HS group size is small, sampling of parental alleles in kinship analyses are limited and kinship assignments less accurate.
- Estimates of pedigree-based inbreeding for individuals and families do not take into account ancestral inbreeding (inbreeding that resulted from co-ancestry between spawner pairs involving G2 or earlier generation Salmon).
- Estimates of pedigree-based inbreeding, and all other metrics of retention of founder parent genetic variation over time, will, to some extent, be impacted by inaccuracies associated with original kinship assignments.
- Estimates of rates of loss of genetic variation based on pedigree metrics, indicators of levels of molecular genetic variation, and effective population size all reflect effects of stochastic processes only (genetic drift); possible loss due to the effects of directional selection for captive conditions are not considered.
- Production, releases, and collections of STW River wild-exposed parr groups are carried out primarily for LGB purposes, not research, resulting in sub-optimal experimental design and creating statistical challenges in testing for differences between treatment sub-group types. For example, average family sample sizes in wild-exposed parr collections are small (fewer

than three individuals), and the number of crosses associated with different cross types (e.g., captive-reared versus wild-exposed) are not always large and balanced.

- Genetic differences among families may have contributed to between-treatment differences reported. The magnitude of this concern is expected to scale at a rate inversely proportionate to the number of crosses associated with each trait type (e.g., the number of captive-reared crosses in comparisons of performance of offspring of wild-exposed versus offspring of captive-reared parents).
- Some findings are based on results from one or two brood years or year class groups, decreasing the generality of results observed.
- Investigations into overall program effects (numbers of cycles of captive breeding and rearing), involved families that differ by a maximum of approximately one generation (families generally consisted of G2 to G3 generation individuals). The magnitude of divergence in trait performance documented under-reports cumulative effects of overall or multigenerational (G0 to G3) captive breeding and rearing on STW River program Salmon.
- Estimates of survival of cross types in the wild, from release to Age 2, and possibly release to Age 1, are based on numbers of family representatives captured via electrofishing at that age; differential emigration out of the release site across families (and especially between treatment types) may impact estimates of effects of different predictor variables analyzed here (e.g., parental rearing environment, European ancestry, etc.).
- Allele frequency distributions between European and North American Salmon at the microsatellite loci for which information are available are partially overlapping; some larger European alleles cannot be distinguished from some shorter North American variants.
- Allele frequency information for any and all farm Salmon sources at the microsatellite loci analyzed here are unavailable.
- Allele frequency information from early (pre-aquaculture) wild Salmon populations from outer Bay of Fundy are limited, which impacts assessments of whether individual short microsatellite alleles observed in recently obtained samples from this group are rare North American variants or recently introduced European alleles.
- The cross-locus prevalence of short European-type alleles within individuals declines sharply with each generational backcross of hybrids to North American Salmon, decreasing the chances of detecting European/iBoF hybrids in wild Salmon without information from additional informative loci.
- Low levels of genetic differentiation between Salmon from the SJR (the reported origin of local source farm Salmon in the area) and iBoF (particularly those of the Chignecto Bay) make identification of putative SJR origin Salmon (either local SJR origin farm Salmon or wild SJR strays) collected from iBoF rivers, using individual assignment tests and available marker data, difficult.
- Unknown origin Salmon collected in the iBoF that unambiguously assign to available oBoF baseline sample collections may either be SJR-origin farm Salmon, or wild SJR strays; assignment tests alone cannot, given available source population information and microsatellite marker data, distinguish between the two. It will be challenging to determine whether iBoF/oBoF F1 hybrids observed in rivers of the iBoF are offspring of an iBoF parent spawning with either a SJR-origin farm Salmon escape or a wild SJR stray.
- The smolt to adult return rates for the GAK River below White Rock Dam should be considered minimum estimates as the potential impacts from the hydro operations have not

been adequately quantified. Downstream bypass efficiency estimates at White Rock Dam have averaged 42.7% (ranging from 16.5 to 66.7%), which indicates a significant proportion of the emigrating smolts is exposed to the negative effects of passing through turbines. As well, upstream migration of returning adults is thought to be negatively affected by low flows in the section of river below the pool and weir fishway during the normal upstream migration period. This uncertainty means the biological data needed to build models to inform managers on population viability and recovery may only be available for the data rich BSR.

- To date, most of the monitoring and evaluation of the LGB releases, both freshwater and marine survivals, have focused on the BSR and GAK rivers. With the exception of the two recent electrofishing surveys, very little effort has been dedicated to monitoring other iBoF rivers, including those have received supplementation releases of all life stages including adult releases.

CONCLUSIONS AND ADVICE

1) At present, there is little potential for natural selection to maintain or improve at-sea survival under current (and possibly changing) marine conditions. For natural selection to impact or improve smolt-to-adult survival of iBoF LGB Atlantic Salmon, a sufficient number of smolt must exit a given iBoF river for a small to moderate number of adults to return to either reproduce in native river habitat, or be incorporated into LGB populations directly as spawners. The likely number of adult returns to individual rivers in the iBoF (0 to 10 in most years with the exception of the Big Salmon River) is probably too low for natural selection to be very effective at maintaining or increasing survival under current and possibly changing marine conditions.

Concerns over the origins of 'in river' produced smolt on the BSR, and decisions to not spawn Salmon that cannot be identified as native BSR in origin, could exacerbate the situation; 'in river' produced smolt collected on the BSR are currently the only source of genes potentially adapted to current marine conditions in the iBoF LGB program (along with, typically, approximately six adult returns to the GAK each year).

Increases in numbers of out-migrating smolt to a level that would result in sufficient numbers (at least several dozen) of yearly returns to supported rivers of the iBoF could help to mitigate these concerns.

There are several possible ways to increase the number of out-migrating smolt, including a) the use of hatcheries to bypass high early juvenile mortality to directly produce large numbers of smolts for spring release, b) increase the number of fry or parr released so that surviving smolt is considerably higher than observed today, c) increase the number of mature adults released so that egg deposition in iBoF rivers is sufficiently high to result in a large number of surviving smolt, and d) increase the freshwater 'in river' survival of released eggs/fry/parr (and/or spawning success of released adults).

In general, smolt-to-adult survival of hatchery produced Salmon generally decreases as the time spent in captivity increases; hatchery produced smolt are expected to exhibit lower survival at sea than hatchery produced parr, and hatchery produced parr are expected to exhibit lower survival than hatchery produced fry. In the iBoF, return rates of hatchery-produced smolt may be very low; thousands of hatchery produced smolt released into the BSR in recent years resulted in no detectable returns. In other words, hatchery production of smolt (a, above) is not expected to be effective and is not recommended at this time.

Simply increasing production of hatchery-origin fry (b, above) or released adults (c, above) is expected to yield, at best, a proportionate increase in numbers of out-migrating smolt (providing increased numbers of fry does not lead to greater inter-individual competition in freshwater

habitat, and density dependent survival). In other words, given current very low marine survival, even a doubling of the number of crosses and released fry (or released adults) is unlikely to result in the needed increase in the number of adult returns.

However, it is possible that a marked increase the number of out-migrating smolt, and the number of expected adult returns, can be achieved using a combination of strategies (b) to (d) described above. Research provided in Harvie et al. (2018) provides insight into variables (e.g., female age at spawning) likely to increase survival from release to at least Age 1. Additional research is needed to identify other variables (under the control of managers) that might further increase freshwater survival of early juveniles and/or spawning success of released adults (e.g., see Berejikian et al. 2003).

2) Reduce, where possible, suspected European ancestry in all three iBoF LGB populations, and minimize future introgression of new European genes into the BSR LGB population.

Stewiacke River LGB population: Initial introgression of suspect European farm genes into the STW River LGB population, involving G0 founders, was limited, and ongoing efforts to detect and remove descendants has minimized, to some extent, European ancestry in this population. Additionally, essentially zero new founders (post 2001) have been collected and incorporated into the STW LGB population. Removal of most/all remaining European ancestry in the STW LGB population should be possible with minimal additional loss of native STW River genes and is recommended. See O'Reilly et al. (2018) for information on which of the five broodstock sets of captive-reared and wild-exposed broodstock groups still contain European farm ancestry.

Gaspereau River LGB population: Initial introgression of suspect European farm genes into the GAK River LGB population, involving G0 founders, was slightly more extensive, and ongoing collection and use of "in-river" produced smolt collected at White Rock Dam (in later years) may have led to more introgression of European genes into this LGB population. Removal of most/all remaining European ancestry in the GAK River LGB population may not be possible without losing unacceptable amounts of GAK River founder variation. Additional analyses should be carried out to determine the relative costs and benefits of culling existing broodstock for European ancestry. However, regardless of the outcome of these analyses, new potential founders, captured as either adults or smolts at the White Rock facility, should be screened prior to spawning in the LGB program.

Big Salmon River LGB population: Initial introgression of suspect European farm genes into the BSR River LGB population, involving initial early G0 founders, appears to have been minimal. However, many smolt captured on the river between 2003 and 2015, and incorporated into the LGB population, exhibited short European type alleles at 1 to 4 loci, and some or all may be descendants of earlier European farm Salmon. The prevalence of European farm genes in existing BSR River broodstock is likely too extensive to remove without extensive loss of BSR River founder genetic variation. However, future introgression of additional putative European farm Salmon genes (and North American farm Salmon genes) into the BSR LGB gene pool should be minimized by excluding as potential LGB parents any "wild collected" smolt not produced by either a known BSR LGB cross or "known" BSR-origin parents spawning in the BSR river.

The implications of this decision, in addition to minimizing rates of introgression of European genes into the BSR LGB population, include (i) minimizing introgression of genes produced by "wild spawning" parents into the BSR LGB gene pool, potentially increasing rates of domestication, (ii) minimizing incorporation of genes that survived current marine conditions in the BSR LGB population, thereby potentially reducing future marine survival of BSR LGB Salmon releases.

A subset of suspect initial G0 European farm Salmon hybrids, from each all three river populations, should be analyzed at Ssa1 and the mitochondrial ND1 SNP sites 4079 and 4424 to further test hypotheses of European ancestry.

3) Increase the number of Stewiacke River wild-exposed parr that are genotyped and pedigreed. Mean family size in genotyped sets of wild-exposed parr are quite small (<3.0). Increasing the total number of wild-exposed parr genotyped each year from the current 250-300 to a minimum of 500 would increase mean family size and improve the ability to detect differences between treatment groups in the future (improving the quality of the science and the potential for adaptive management of the iBoF LGB program). Diverting genotyping capacity from captive-reared to wild-exposed offspring groups would allow for this increase without any additional funding; this would also be compatible with other recommendations (see below) to transition from the spawning of captive-reared and wild-exposed Salmon to spawning exclusively wild-exposed Salmon only.

4) Continue shifting from the use of captive-reared and wild-exposed Salmon to greater use of wild-exposed Salmon. Originally, in all three programs, a similar number of captive-reared and wild-exposed Salmon were spawned each year. Over time, there has been a shift towards the preferential spawning of wild-exposed over captive-reared siblings, such that most Salmon spawned for the LGB program in a given year were exposed to wild-river conditions as juveniles. Given results from Evans et al. (2014) on preferential survival of offspring of wild-exposed versus captive-reared parents, and converging estimates of variance in family size in captive-reared versus wild-exposed offspring groups over time (across years 2002-2010), further movement in this direction is recommended. Exclusive spawning of wild-exposed Salmon in the STW River program is recommended, and captive-reared counterparts should only be reared for contingency purposes, until wild-exposed siblings have been captured and shown to successfully transition to hatchery conditions. Captive-reared Salmon can be released in the spring of their second year (Age 2). Existing efforts to continue to spawn wild-exposed Salmon over captive-reared counterparts in the BSR and GAK programs should continue.

5) Use of Ranked Mean Kinship broodstock management algorithms, delayed reproduction (from Age 4 to exclusively Age 5), and at least partial isolation of brood year groups in the management of the STW River LGB population are recommended. The current broodstock management regime put in place for STW River population (Ranked Mean Kinship, delayed maturation, isolation of brood years with limited gene flow where required) is recommended going forward for this group of iBoF Atlantic Salmon. Delaying reproduction will result in fewer cycles of captive breeding and rearing, and research carried out in Harvie et al. (2018) demonstrates that negative effects of spawning males and females at Age 5 are not expected. Isolating brood year groups, and the associated maintenance of large effective population sizes (>200 but <500) may reduce rates of adaptation to captivity. However, populations should be monitored for signs of inbreeding depression, and levels of cumulative inbreeding should be assessed from time-to-time. Low levels of gene flow amongst sub-populations in the future would be encouraged if warranted. Published studies elsewhere and research carried out in O'Reilly et al. (2018) on iBoF Atlantic Salmon (e.g., under relevant conditions, including levels of family structuring in the G0 generation) indicate that Ranked Mean Kinship breeding algorithms should be effective in minimizing rates of loss of genetic variation through time, at least through to the G10 generation and likely beyond this.

6) The STW River LGB population should be highly prioritized for conservation. Stewiacke River Salmon exhibit (1) unique genetically-based phenotypic traits (small size at age and low compensatory growth), (2) high frequencies of the iBoF mitochondrial DNA clade 1-3 (Verspoor et al. 2002; Verspoor et al. 2005; O'Reilly et al. 2018) (similar to that observed in archival iBoF sample collections), and (3) little influence of European farm or Saint John River Salmon

ancestry. The current population may still retain many of the genetic characteristics of the original iBoF assemblage of populations.

7) Evaluate the feasibility of modifying the current GAK River program to include some or all of the recent modifications put in place for the STW River program. Salmon from the GAK River are currently managed using MKA methods, and individuals are spawned, primarily, in either their fourth or fifth years; this permits extensive gene flow between year class groups and results in very large generational effective population size and high potential rates of adaptation to captivity. Use of Ranked Mean Kinship may reduce rates of loss of genetic variation, and spawning Salmon exclusively in year 5 would both reduce the number of cycles of captive breeding and rearing and isolate brood years (see #6 above for details). However, the reproductive biology of this population (alternate year spawners) may be incompatible with this management regime, though there may be ways to mitigate each. Additional analyses should be carried out to determine the feasibility of adopting one or all of these changes.

8) The BSR breeding program (MKA and spawning in year 4 or 5) should continue as is, at least for now. Levels of genetic variation observed in BSR have not declined in recent years and may have increased. Additionally, the observation of high and variable survival of BSR adults reared at Mactaquac between Ages 4 and 5 in the past (primarily a result of water-borne pathogens found in river available at the facility) are incompatible with the strategy of delaying reproduction until Age 5. However, if conditions change in the future (reduced introgression of genes from non-local sources and later reductions in levels of within population genetic variation and/or reductions in mortality from Age 4 to Age 5), modifications to breeding protocols should be considered.

9) Additional cryopreservation of milt is recommended. If possible, milt from additional males should be cryopreserved, before additional genetic change occurs. Cryopreserved milt may be used later to partially restore genetic characteristics of iBoF Atlantic Salmon.

10) There are several possible paths to maintaining large effective population size in iBoF LGB programs, each involving variable levels of expenditure of different resources; the optimal approach should consider the scarcity of each resource at a particular point in time. Regardless of which approach is chosen to achieve large effective population size, some level of molecular genetic monitoring is important for on-going evaluation of the prescribed conservation program, and to ensure that rates of loss of genetic variation and accumulation of inbreeding are not excessive.

11) Consideration should be given to evaluating and using new molecular genetic markers in the management and conservation of iBoF Atlantic Salmon (Single Nucleotide Polymorphisms, or SNPs). This information can be used to maintain pedigrees (via parentage assignment), enable other research, and, possibly, more effective adaptive management of the iBoF LGB and supplementation programs. Examples of research include a) identification of markers to detect (and cull) European and possibly North American farm ancestry, b) genomic-based studies of the effects of captive breeding and rearing, and (potentially) c) identification of genes or chromosomal regions associated with high marine survival.

12) Consideration should be given to expanding the field and genetic programs on the STW River to allow for monitoring life stage survivals similar to the BSR and GAK programs. Currently, the majority of the data collected for assessing the STW LGB program is related to monitoring genetic diversity. The smolt assessment program that involves the operation of a RST initiated by Mikaw Conservation Group and assisted by DFO Science is a positive start. Expanding the STW program would provide another river besides the BSR river to assess population viability. Consideration should be given to further investigate the existing dataset of

in-river LGB survival from the BSR, i.e. examination of parental origin (captive and wild-exposed), environment variables, stocking density and release date on LGB in-river survivals.

13) A directed broadscale electrofishing survey designed to investigate the return of sea run spawners in iBoF rivers every 10 years seems appropriate. Historical broadscale electrofishing surveys were done in 2000, 2002, 2003 and 2014. The interest for the 2014 survey was to update juvenile Salmon presence/absence and abundance/origin in the non-LGB supported iBoF rivers for the iBoF Recovery Program review.

14) Consider shifting some of the focus of the inner Bay of Fundy Recovery program from maintenance of genetic variation to re-establishment of wild self-sustaining populations. Much of the efforts thus far have been directed towards the conservation of genetic variation, or to achieving the first part of Objective 1 in the iBoF Atlantic Salmon Recovery Strategy (Conserve iBoF Salmon genetic characteristics and re-establish self-sustaining populations to iBoF rivers). There may be value in directing more effort towards the second half of this objective (re-establishing self-sustaining populations to iBoF rivers). For example, the numbers of juveniles released in the supplementation program could be increased.

If a shift in focus of the iBoF Recovery program from maintenance of genetic variation to re-establishment of wild self-sustaining populations were to be considered, the extent to which resources ought to be redirected should reflect both costs (e.g., reduced efficacy of conserving genetic variation) and benefits (e.g., the likelihood of making progress toward re-establishing self-sustaining populations, given current marine conditions).

Re-establishment of self-sustaining populations may also be facilitated by increasing performance (including survival) of juveniles already produced through the supplementation program. Harvie et al. (2018) provide some insight into measures that might increase size-at-age (e.g., alteration of the parental rearing environment) and others that appear to increase survival in the wild to at least Age 1 (increasing parent age and egg size). More research is needed to identify additional variables that might further increase offspring survival in both freshwater and marine phases of the species' life cycle.

SOURCES OF INFORMATION

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